

5. Computational Infrastructure for Imaging



There is a strong need for computational and informatics techniques that can meet the imaging challenges in data analysis, modeling, and distributed access and archiving. We expect that model systems and field studies will generate massive amounts of multi-spectral spatiotemporal image data at multiple scales. Figure 5.1 shows examples of different data modalities (imaging technologies) that may be used alone or as complements for investigating living systems at different scales.

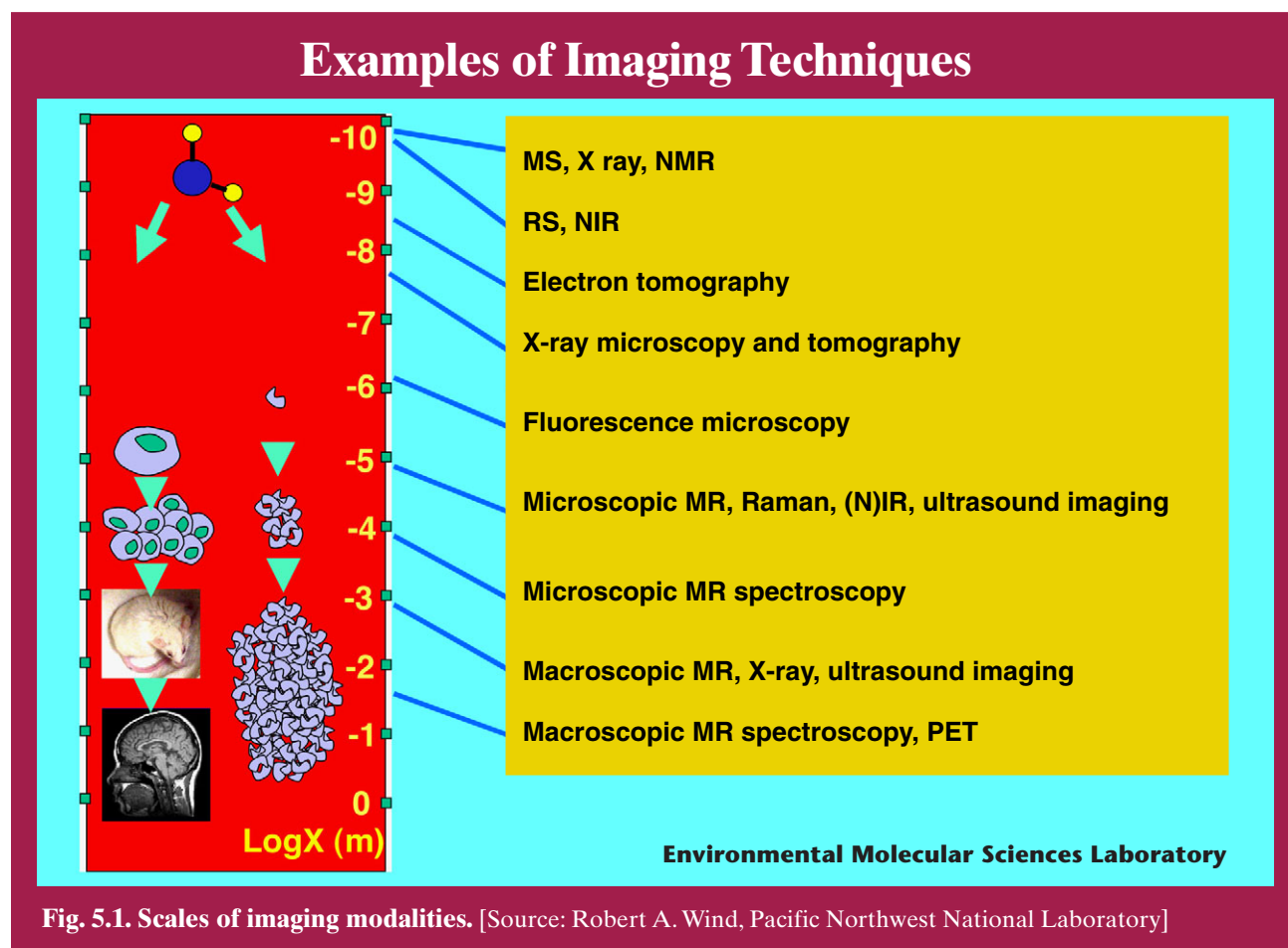
A particularly valuable goal for the future is a computing environment that allows scientists to acquire data, archive a set of data, annotate and link the data from different modalities, find relevant computational tools on the Internet,

compute various representations of these data for quantitative analysis, and compare observational data to simulated data when possible.

Issues

Significant barriers to the use of computational resources for imaging studies include the following:

- Useful computational tools in various forms in laboratories around the country that are not easily accessible by community researchers.
- Diverse set of algorithms and software tools for solving a specific problem that does not characterize reliability and performance behavior.



- Lack of connectivity among instrumentation, computing infrastructure, and diverse databases.
- Lack of algorithms for modeling and inverse problem solving.
- Lack of user training in using advanced scientific tools.

In this context, we propose investments in several areas. Some of these research activities can be coordinated with the DOE Office of Science's Mathematical, Information, and Computational Sciences (MICS) office. MICS actively funds basic research in scientific data transport and analysis, information visualization, and computational physics; examples are adaptive mesh refinement with embedded boundaries and libraries based on general partial differential equations (PDE) for high-performance computing.

Technology Needs

Discussed below are technologies needed for the computational infrastructure for imaging, including data, compute, user, and infrastructure services.

Data Services

These services include data annotation, publishing, subscription, and retrieval. Data should have self-describing annotations, and users should be able to publish their data against an existing study or project topic. At the same time, users should be able to subscribe to the data and corresponding annotations generated by other researchers. There could be several mirror sites for archiving images, but users could have a private copy of their own data. The archive could be searched against user-defined annotations as well as computed features; for example, to collect all transmission electron microscopy (TEM) images of *Geobacter* in the GTL community or search for an example of biofilm at a specific state of its evolution.

Compute Services

These general computing services, including data preprocessing, data fusion, data and quantitative analysis, modeling and simulation, and visualization, should be available to users in a seamless fashion. The capabilities of each area should be

integrated to allow users to move from one to the next without having to invest considerable time in learning a new method of doing the work.

Preprocessing

Image data are generated by various instruments used to study biological structures, from individual proteins to complex microbial communities. Up-front data processing is needed to improve data quality for subsequent visualization and analysis. In some cases, this preprocessing step will be specific to the type of imaging instrument used in the experiment. However, some preprocessing steps will be generally applicable to many modalities.

Filtering. Filtering algorithms are needed to eliminate or reduce noise (e.g., random and spatially correlated) or alter the frequency content of the image (e.g., smoothing, sharpening, and edge detection). In the case of image noise, different types are inherent to specific imaging instruments [e.g., speckle noise in magnetic resonance imaging (MRI) and X-ray tomography]. Filters that can be modeled after the physics of the instrument noise source have the best chance of being the most effective. Existing and new filtering algorithms should be extended to 3D so instruments capable of 3D imaging can benefit from this technology. In addition, improved 3D filters are needed that suppress or enhance morphological structures of a particular object (e.g., a cell) for subsequent segmentation and recognition purposes.

Data Reduction. A substantial imaging challenge in the GTL program is how to handle the vast amounts of image data generated during experiments. For example, a 3D multispectral image data set acquired at different times during a single experiment can be extremely large (tens of gigabytes), so the task of analyzing, storing, and sharing all this data is significant. One way to address the problem is to apply new and intelligent data-reduction technologies to retain only the data critical for subsequent fusion and analysis. Two areas that address this are image-data compression and dimensionality reduction:

- **Compression.** Technologies for image-data compression must be developed in collaboration with biologists to ensure that important

information is not compromised. New content-based data-compression technologies are needed that, for example, highly compress noncritical regions of the image using lossy compression algorithms but apply loss-less compression strategies to information-rich image regions. Fast up-front image-analysis algorithms are required to differentiate these critical image regions from noncritical regions. Other new and creative compression strategies that allow large image-data sets to be more easily stored and efficiently transmitted over the Internet are essential for accelerating GTL program science.

- **Dimensionality Reduction.** Another way to reduce the amount of image data is to convert to an alternate representation in which fewer dimensions are required to describe the important aspects. In a multispectral data set, new approaches might select only the spectral components containing information important for the application at hand. For a temporal study, this could involve change-detection algorithms that allow certain time sequences to be excluded if no significant biological activity is occurring. Other approaches would be to apply principal component-analysis strategies to the data so that only the major modes of variation would be captured, thereby reducing the data set's overall dimensions. The main goal here is to reduce the data set but ensure that it still captures the experiment's important essence.
- **Missing-Data Interpolation.** In many instances, the image-data set from a given instrument will contain incomplete or corrupt data. Examples of this follow:
 - Gaps in 3D data sets from limited-view angle tomography or MRI.
 - Missing or corrupt slices from a 3D data set (TEM, confocal).
 - Photobleaching and detector saturation in fluorescence microscopy.

Missing or corrupt data typically are found only after manual examination of image data and are then corrected using a combination of manual

and semiautomated techniques. These gaps in the valid data set can create significant problems for automated data-analysis algorithms that are applied downstream for such purposes as object segmentation and quantification. New approaches must be developed that (1) automatically detect these missing or corrupt regions of data and (2) either flag data so that it is ignored in any downstream processing or accurately interpolate new data to replace gaps.

Data Fusion

Biological image data can be obtained from many sources or modalities such as Raman spectroscopy, confocal microscopy, and MRI. Using these different modalities, image data of the same subject can be obtained from multiple sensors. These data sets could have vastly different, often complementary, content. To fully exploit information distributed among data sets, techniques for data fusion will be necessary. To effectively combine individual data-set information, relationships between image-data aspects relating to the subject's physical attributes should be defined for the various image sources. Once these relationships are defined, they can then be used to map all images to a common representation (i.e., coregister the images to a common scale and orientation). Registration can be based on landmarks and include global or local warping of images obtained from different modalities. Global alignment often involves estimating parameters of an affine transform. Localized alignment corrects for localized distortion typical of many scientific instruments.

Data and Quantitative Analysis

This includes object detection, segmentation, motion tracking, feature-based query, matching and comparative analysis, multivariate data analysis, and classification. The problem's important aspect is the need to extract meaning from the image data. Many times this part is overlooked and left to users to determine their own approaches to get meaningful results. Data and quantitative analysis should be integrated in the overall system as well as in other areas. However, research often is still needed to develop these "tools" to help users quantify their data.

Object Detection. Counting is often necessary to detect the presence of objects of interest, record their position, and learn something about the underlying organization. Object detection can be based on simple correlation to a more elaborate model- and appearance-based approach.

Segmentation. A critical aspect of image analysis is segmentation (Fig. 5.2), which aims to delineate objects of interest and their individual compartments so that morphological features, protein expression, and physiological responses can be characterized on a cell-by-cell basis from each image and collection of such images. An important aspect of segmentation is its robustness and tuning for parameters. A new algorithm may need to be developed for a specific data set. Current computational techniques, however, appear suitable for a large class of fluorescent imaging microscopy. Interactive techniques (e.g., “snakes”) will be provided for processing small sets of data.

Motion Tracking. Time-lapse microscopy has the potential of producing massive amounts of data (Fig. 5.3). A typical time-varying 2D experiment can generate 12 MB of data per minute at 30-s sampling intervals (1- by 1-k images at 12 bits per pixel at three different spectrums). Bacteria need to be detected and tracked. The ability to track a community of bacteria enables a more basic understanding of pattern formation and organization under a number of different conditions.

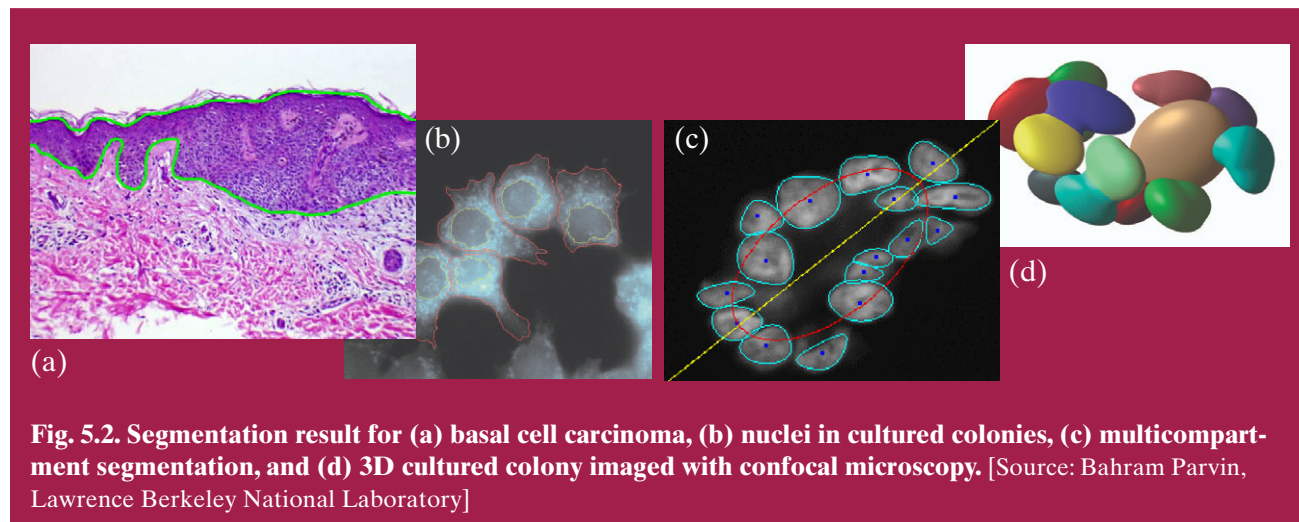
Feature-Based Query. Searching an image database against a reference texture and intensity often is desirable. For example, the fluorescence

texture of a biofilm indicates its evolution and may be used as a template texture of interest to search for similar textures that may have other properties.

Matching and Comparative Analysis. Experiments are often designed to compare function, physiology, and behavior under different experimental conditions for same species, genetically modified species, or different species. Techniques must be developed for comparing experimental data in a systematic way, which may include sensitivity analysis.

Multivariate Data Analysis. As technology advances to interrogate complex biological systems, images often contain more than one variable of interest and are known as multivariate data. This is in contrast to univariate images in which only one variable, for instance a spatial map of Ca^{++} ion concentration, is obtained. Examples of multivariate data are multispectral and hyperspectral images such as Fourier transform infrared spectroscopy (called FTIR) and MRI. Data sets or images combined with temporal and environmental parameter information (i.e., temperature and treatment) also can be multivariate.

Multivariate data sets are often enormous, containing more than tens of thousands of pixels. A researcher interested in determining qualitative and quantitative relationships in the data can no longer simply look for the answers visually. Instead, sophisticated multivariate analysis algorithms are needed to extract useful information



from multivariate image data. These algorithms use the variance in these huge data sets to determine relationships among variables and generate image-contrast and quantitative-concentration maps. Some very powerful multivariate algorithms such as principal-components analysis, factor analysis, least-squares regression, and multivariate curve resolution need to be applied, optimized, and automated for biological data and images. In addition, new, robust algorithms must be developed to more fully characterize the data in a quantitative fashion. These algorithms may include improved methods to remove signals from contaminants, background variation, and instrument or experimental artifacts, all of which are commonly found when cells and signaling events are probed in situ and in vivo.

Classification. Classifying groups of cells and structures, ideally in real time and in multicellular, heterogeneous systems is important. For example, there is a need to classify bacterial cells based on type while monitoring their behavior in culture. Although techniques such as hierarchical cluster analysis and discriminant analysis have been shown to provide classification of biological tissues and species, further advancement in this area is needed. Techniques available from other

disciplines may be well suited for biological-data classification. These as well as novel algorithms should be investigated and adapted for real-time image classification of cellular species and events.

Modeling and Simulation

Providing a modeling and simulation component to analyze, interpret, and predict biology data and experiments represents an approach to helping make biology a more quantitative science. Modeling is the mathematical definition and algorithmic implementation of a biological process. Simulation is a computer realization of a biology experiment. Taken together, modeling and simulation constitute an approach to doing “virtual biology” experiments. A modeling and simulation capability can take many different forms, from simple reaction network models to full temporal and spatially resolved models.

Image data from biology experiments are useful for the modeling and simulation community as a source of data for parameter estimation (e.g., chemical reaction rates and diffusion coefficients), initial and boundary conditions (e.g., velocity and temperature), geometric feature data, model-verification data, and simulation-validation data. Different types of image data from a single biology system may be combined to form the basis of a biology modeling and simulation “virtual experiment.” Some examples follow:

- Nuclear magnetic resonance (NMR) and spectral imaging techniques show chemical reaction rate data.
- NMR data can provide distributed maps of component velocity and diffusion coefficients.
- Geometric surfaces defined by feature extraction and reconstruction algorithms from volume image data form a source of geometry on which volume meshes can be used for discrete time- and space-dependent integration methods including finite elements, volume, and difference algorithms.

The sources of this type of image data include confocal microscopy, NMR, X ray, and electron tomography.

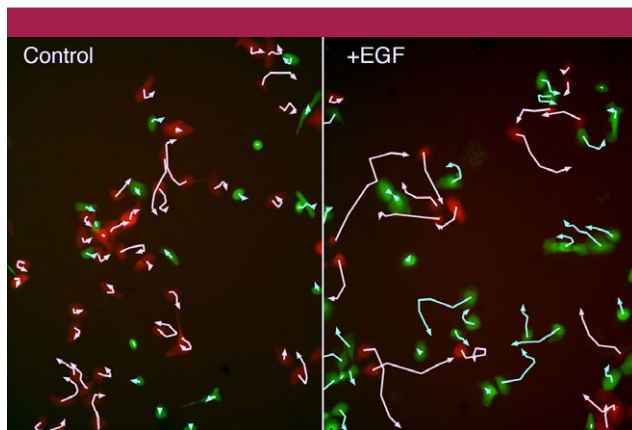


Fig. 5.3. Control cells (green) and engineered autocrine cells (orange) mixed overnight and followed for 4 h by two-color fluorescence time-lapse microscopy. Shown are the initial images overlaid with trajectories marked at 40-min intervals. Cells were either in the absence (left) or presence (right) of 2-nM exogenous growth factor (EGF). [Source: Reproduced from the *Journal of Cell Biology* (2001), **155**(7), 1123–28 by copyright permission of The Rockefeller University Press.]

Figure 5.4 represents the series of steps to transform a 3D digital volume image (made up of a stack of 2D TEM images) into a computed 3D geometric mesh.

Visualization

Improved data- and information-visualization techniques will be necessary to assist researchers in their overall understanding of the biological system (Fig. 5.5). A number of systems already can accommodate 3D volume data with reasonable speeds. Data transfer among visualization methods will be important to help researchers present their ideas.

Where appropriate, visualization systems should be used and seamlessly incorporated into the computational environment. Although volume rendering is a standard tool for viewing the quality of raw 3D data, surface rendering often is used with segmentation to evaluate the quality of automated analysis. Triangulation is appropriate for visualization, but it is not the tool of choice for 3D geometric analysis of surfaces.

User Services

This is a general classification of services for software training, design, and availability that need to be provided at all levels of implementation. These services include documentation and tutorials, open-source software, self-describing data, location of application-specific data-analysis tools, and remote instrumentation (Figs. 5.6 and 5.7).

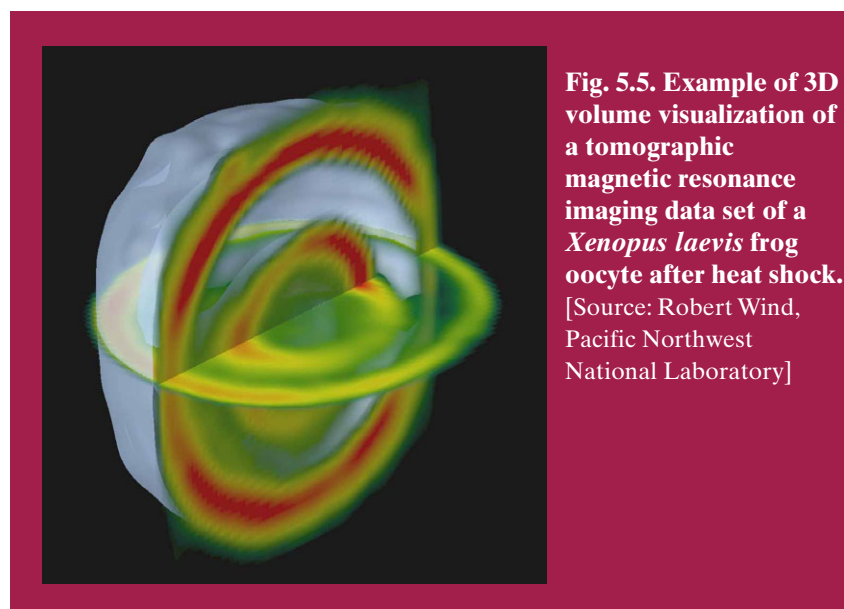
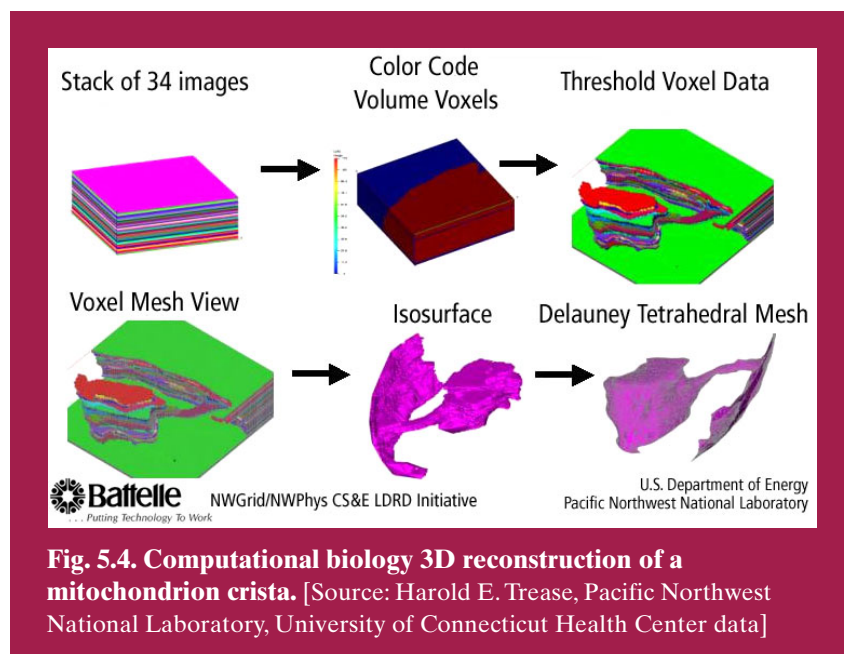
Documentation and Tutorials

All services should be documented with tutorials and examples to enable users to directly access a resource. Workshops must be established to learn

more about user needs. Experience indicates that one-to-one interaction is often necessary for successful application of a particular tool. Part of the workshop series should include classes with homework and exercises that will be modeled after the current coursework taught for comparative genomics.

Open Source

All computational tools developed for GTL should be open source to enable users to process their data on the desktop.



Self-Describing Data

Data should be annotated with a markup language so Web pages can be generated automatically from user-defined descriptions. The markup language will simplify exchange and distribution of data among multiple institutions.

Locating Application-Specific Data-Analysis Tools

Stepping through a large data set and processing it for quantitative analysis is often necessary. An example is quantifying protein expression in cells that reside at a specific location in tissues. A typical study may include several hundred images with 5 to 100 cells per image. The user should be

able to locate an appropriate analysis tool or set of tools based on this level of problem statement.

Remote Instrumentation

Remote instrumentation satisfies two requirements: (1) it enables researchers to share a unique instrument for data collection, and (2) it couples data acquisition with computation and data services to streamline the process.

Infrastructure Services and Computing Resources

The proliferation of imaging methods and analytical approaches has created two significant challenges:

1. Current approaches for analyzing and interpreting image data are still inadequate to meet the special challenges associated with the complex multisensor data sets typical of advanced applications.
2. The numerous analytical-software tools resulting from this research generally are not compatible with each other, a situation that hinders collaboration and more rapid progress—

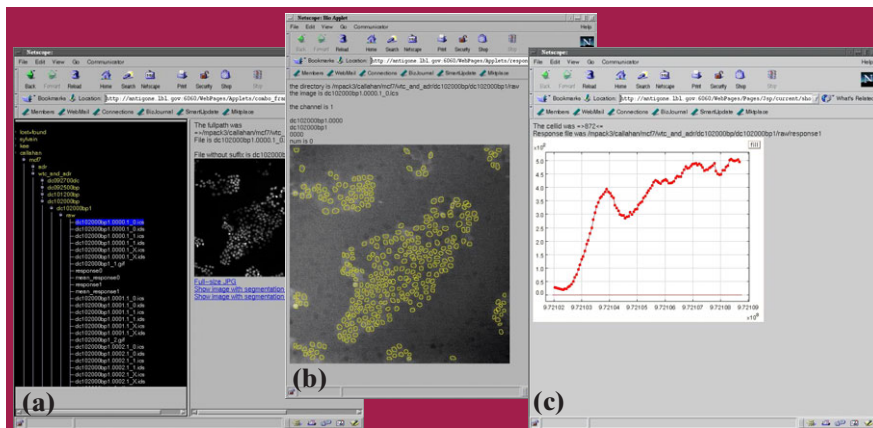
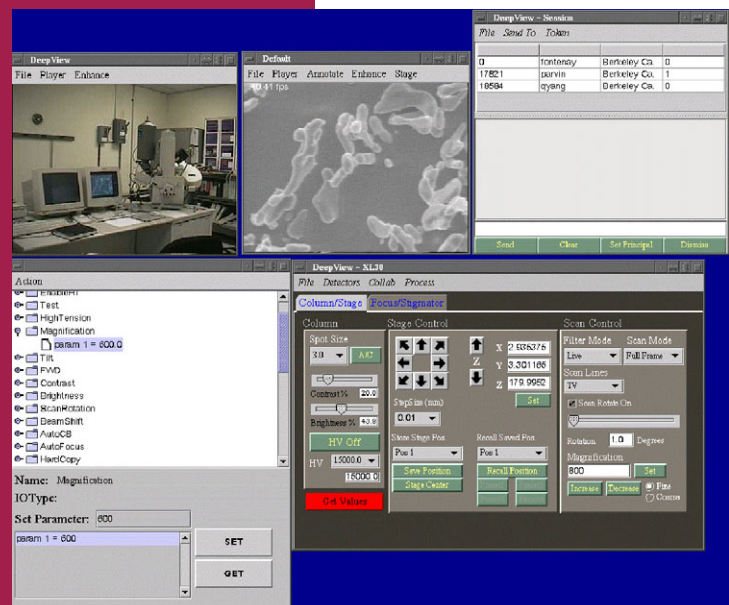


Fig. 5.6. Automated uptake analysis for a population of cells: (a) image and corresponding data model, (b) active page with segmented result, and (c) time-series response of a selected cell from (b). [Source: Bahram Parvin, Lawrence Berkeley National Laboratory]

Fig. 5.7. An example of remote instrumentation for shared collaborative use—the DeepView architecture for remote microscopy funded by MICS. DeepView provides a self-describing framework for defining scientific instruments. This example shows DeepView's front end to a scanning electron microscope at Oak Ridge National Laboratory. [Source: Bahram Parvin, Lawrence Berkeley National Laboratory]



especially across domain boundaries, where radical scientific advances often occur.

Development of a flexible, extensible “architecture” for image-processing and -analysis tools is needed to help researchers collaborate more effectively and gain greater insight into the imagery they study. This architecture would provide a framework of standards, protocols, and

basic utility functions into which new and innovative image-analysis methods could easily be placed and compared with other techniques. The architecture will allow access to heterogeneous databases, leverage current efforts funded under the Scientific Discovery through Advanced Computing (called SciDAC) program, and provide scalable evolution for meeting new scientific requirements (Fig. 5.8).

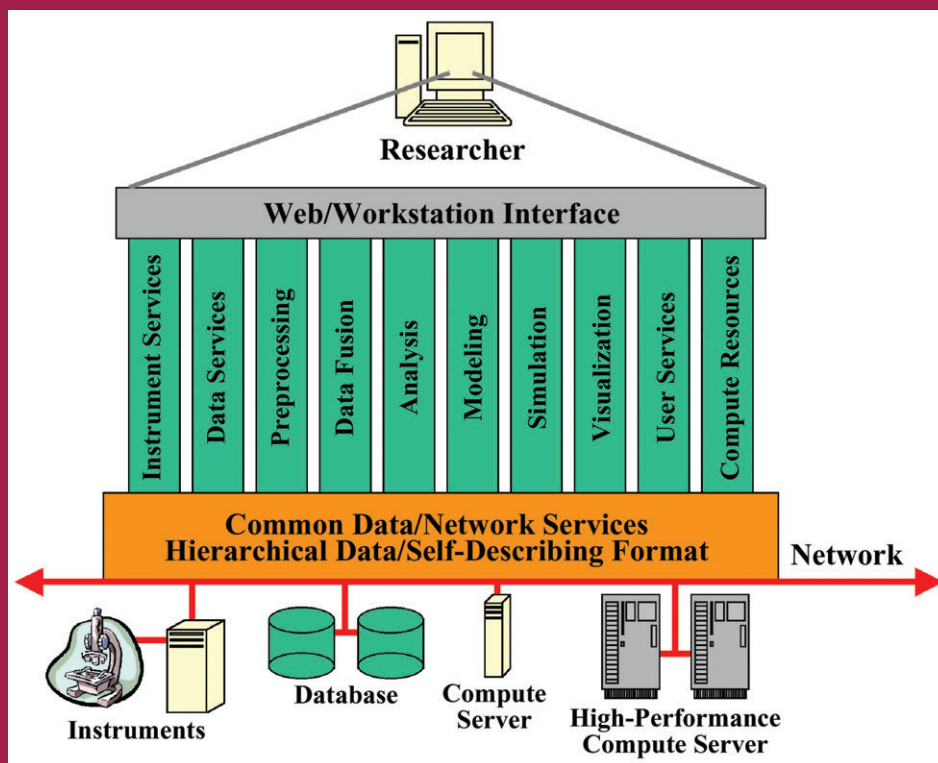


Fig. 5.8. Conceptual vision of the infrastructure for imaging. [Source: Donald R. Jones, Pacific Northwest National Laboratory]

6. Probe Development for Advanced Imaging Methods



Key to all aspects of the GTL program is development of improved and new probes. Fluorescent probes are key reagents for extracting information from cells and tissues. Their use has expanded greatly over 30 years, and a billion-dollar industry is based on fluorescent detection. Approaches for probe development, current status, and needs for new probes are discussed below. Fluorescence technologies contain the most mature examples of probe development at this time, but new probes are needed for other imaging technologies, including infrared (IR), Raman, surface-enhanced Raman particles (SERS), coherent antistokes Raman scattering (CARS), X ray, and magnetic resonance.

The ideal probe for monitoring molecular machines and biological functions has several characteristics. First, it should provide a robust signal that is easily observable above any background present in the sample. Second, it should be unaltered by the means through which it is observed and by the sample into which it is introduced. The probe should be highly selective in recognizing the intended target and have a high mobility to the regions of interest. It should be optically stable, allowing the dynamic processes associated with molecular machines to be monitored over long time scales. Finally, it should be small enough to be easily incorporated without perturbing the system being studied.

Having defined the ideal probe, we must recognize the trade-offs with current probes. For example, organic chromophores are small and can be easily conjugated to a number of biological molecules. However, even the most robust organic chromophores will emit photons for only a few seconds before photobleaching when saturated. At the single-molecule level, this severely limits the amount of time that dynamic processes can be monitored. On the other hand, plasmon-resonant particles (see below) are

extremely bright and do not photobleach; however, their large size (40 to 100 nm) prevents their use in a number of systems.

In addition to optical probes, new density probes are needed for use as fiducials in tomography and as high-resolution labels for individual protein subunits in complexes being characterized or located by transmission electron microscopy (TEM) or X-ray microscopy. These should have high density, chemical specificity, and minimal nonspecific binding.

Applications for Fluorescent Probes

This section addresses the wide range of current and possible applications of fluorescent probes (Fig. 6.1).

Labels and Indicators

Labeling

- DNA and RNA for fluorescence in situ hybridization (FISH), antibodies for immunofluorescence, ligands for drug discovery, and intracellular proteins for studying cell function.

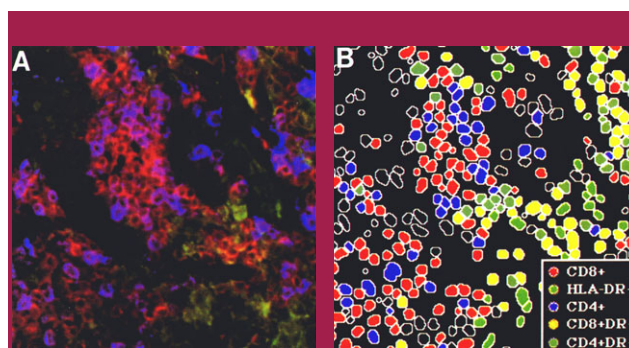


Fig. 6.1. FISH using multiple fluorescently labeled DNA probes. [Source: A. I. Dow et al., "Automatic Multiparameter Fluorescence Imaging for Determining Lymphocyte Phenotype and Activation Status in Melanoma Tissue Sections," *Cytometry* **25**, 71–81. ©1996, Wiley-Liss, Inc. Reproduced with permission of John Wiley & Sons, Inc.]

- Immunofluorescence, diagnostics, gene expression, and DNA sequencing.

Live-cell indicators

- **Ion concentrations.** pH and calcium are the main targets. Most probes for other ions need improvement. Long-wavelength and wavelength-shift probes for ions and other physiological parameters are needed (Fig. 6.2).

Gene expression: Beta lactamase, fluorescent proteins, and fluorescein digalactoside

- **Membrane potential.** Some membrane-permeant probes respond too slowly to determine vesicle and cell potentials. Rapid-

responding probes permit detection of action potentials in neurons. Current probes show relatively small changes in fluorescence response to changes in membrane potential. Improving the fluorescence response would make these probes more useful. Probes that become brighter with integrated neural activity are needed.

Cell and vesicle tracking

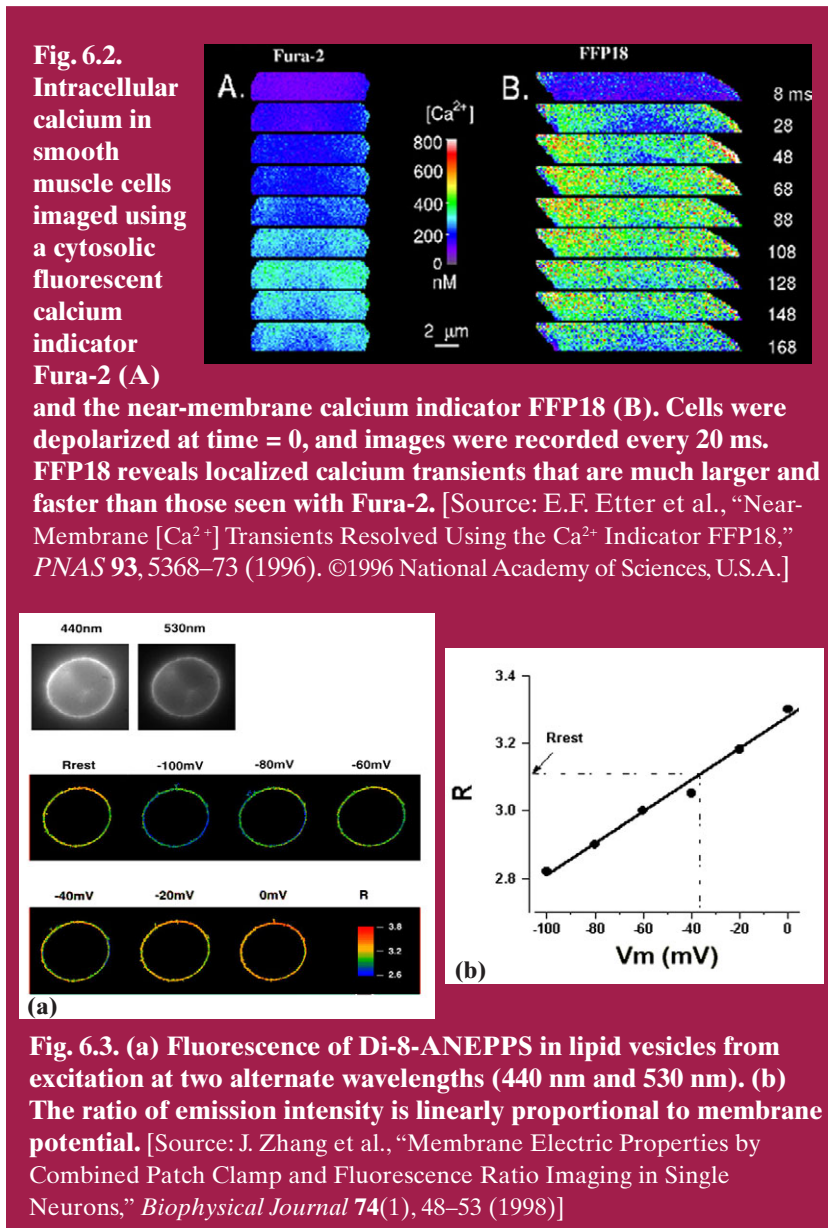
There is a need for near-IR fluorescent tracking dyes that can be used deeper in complex multicellular systems as well as protein-protein interaction and conformational changes. This exciting new area is beginning to grow with the use of

multicolor fluorescent proteins that can be incorporated into the design of biosensors for kinases and other regulatory protein activities. There is a strong need for chemical and genetic methods of introducing multiple fluorescent labels into specific sites on specific proteins so that new cellular sensors can be constructed (Fig. 6.4).

- **Cell- and mitochondrial-energetics indicators.** Other than NADH (nicotinamide adenine dinucleotide), FAD (flavin adenine dinucleotide), and membrane-potential probes, there are no good indicators of energization. Such indicators would be valuable for studying mammalian cells and populations of microorganisms. The need for improved redox potential indicators should be included in this category.

- **Enzyme-activity indicators.** Few probes can indicate the wide range of enzyme activities in cells.

- **Protein-concentration sensors.** Targetable biosensors are needed to indicate the concentration of different regulatory proteins in cells and in the interstitial spaces between cells.



- **Additional cell and tissue properties.** Probes in need of improvements include those for lipid-protein interaction, nitric oxide, pressure-strain, oxygen, and cell health.
- **Membrane dynamics.** Improved probes are required to study dynamics of lipid-protein interactions in membranes (Fig. 6.5).
- **Single-molecule studies.** Ways to tether and orient the probe at particular sites are needed.

Philosophy of Probe Development

A need for specific biological information drives probe development, which entails an understanding of cellular and biochemical systems, instrumentation, and chemistry. The probe chemist uses the theoretical principles of fluorescence and chemistry to design a core chromophore-fluorophore, the core light-absorbing fluorescent

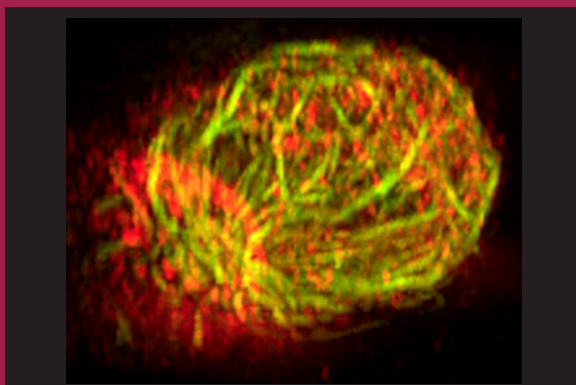


Fig. 6.5. A cytotoxic T cell (CTL) engaging an antigenic target immunostained for tubulin (green) and LFA-1 (red). The target cell in this image is not visible because its microtubules were depolymerized before mixing with CTLs. This image shows the dynamic clustering of LFA-1 into a ring at the target contact site. [Source: Reprinted from J. R. Kuhn and M. Poenie, "Dynamic Polarization of the Microtubule Cytoskeleton During CTL-Mediated Killing," *Immunity* 16, 111–21 (©2002), with permission from Elsevier Science]

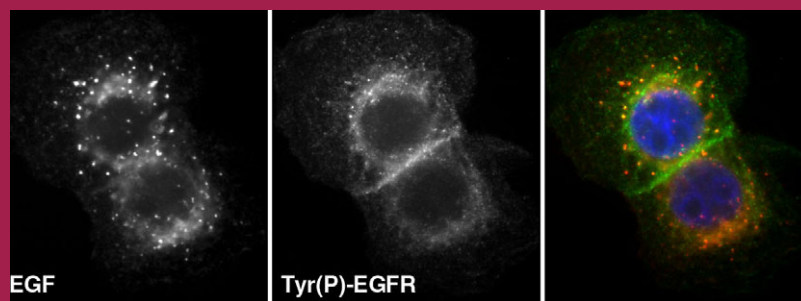


Fig. 6.4. Localizing sites of receptor activation by a juxtacrine ligand. Cells expressing EGF receptors (EGFR) were engineered to also express membrane-anchored EGF. The cells were fixed and stained with an Alexa488-labeled antibody to EGF (left panel) and affinity-purified antibodies to the activated EGFR (middle panel). Strongest receptor activation was seen at sites of cell-cell contact and transport vesicle-containing ligand. [Source: H. Steven Wiley, Pacific Northwest National Laboratory]

component of the probe. The design also must incorporate chemical or genetic modifications that will permit the core fluorophore to function as a probe. At the same time, the probe chemist must be knowledgeable about instrumentation that will be used to extract the probe's information in the biological environment so that it functions optimally. The probe is then ready for testing and use.

Fluorescent Probe Design: Integration of Fluorescence Principles

The power of fluorescence detection lies in the various fluorescence "tricks" that can be used to obtain molecular information. The probe designer and the applications and imaging scientists must be aware of the principles below so probes are properly designed and used (Figs. 6.6 and 6.7).

- | | |
|--|-------------------------------------|
| • Simple signal detection from probes in cells and tissues | • Polarization |
| • Single-molecule detection | • Fluctuation spectroscopy |
| • Multicolor detection | • Surface-resonance effects |
| • Energy transfer | • Time-delay fluorescence detection |
| | • Combinations of the above |

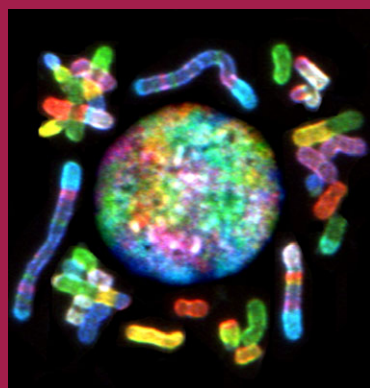


Fig. 6.6. Multicolor staining for different proteins (CD8, CD4, and HLA) allowing different cells to be distinguished in a mixed population.
[Source: Thomas Ried, National Institutes of Health]

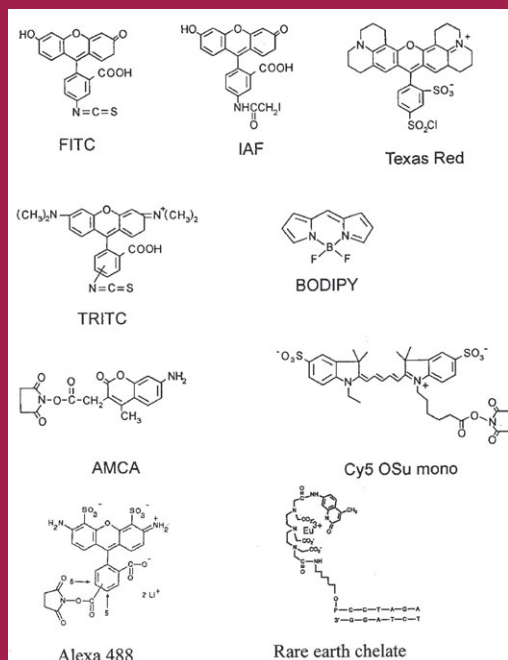


Fig. 6.7. Structures of a number of common fluorophores.
[Source: Alan Waggoner, Carnegie Mellon University]

development of sensitive indicators (see Table 6.1). One of the important features of fluorescent probes is their brightness, which is a function of their extinction coefficient and quantum efficiency, shown in the table for some commonly used probes. As can be seen, a number of these probes are near their theoretical maximum. Other problems needing to be addressed include probe-probe quenching and the presence of other quenching mechanisms. Development of fluorophores with large two-photon absorption cross sections is important but has not received much attention.

Wavelength

Probe absorption must be matched to excitation wavelengths. Overlap of fluorescence peaks in multicolor experiments is limiting. Although quantum dots have narrow emission peaks, smaller organic dyes with narrow peaks also are needed.

Photostability

Photostability is important in most applications. Bright dyes that quickly fade when illuminated are inherently less useful. For many high-throughput applications, the photostability of current probes is adequate. For single-molecule detection and optical sectioning of cells and tissues, however, improvements in

photostability are required. Photostable near-IR fluorophores are particularly needed to extend the range of multicolor fluorescence detection and to provide probes for improved deep imaging in complex multicellular systems (Figs. 6.8–6.12).

This parameter is important in probe design to optimize excitation and light collection while permitting multicolor detection. Different applications have different requirements.

Fluorophores with excited state lifetimes in the 10- to 100-ns range would be useful for autofluorescence and background reduction yet would produce high replication rates for signal acquisition.

Fluorescent Probe Design: Design of the Core Fluorophore

Brightness

Brightness of the fluorophore is essential to overcome background and noise. Generally, the brightness is the numerical product of the light-absorbing power (extinction or cross section) and the quantum yield. We already have some visible and UV fluorophores that are within a factor of 2 to 3 of their maximum potential.

However, we need more of these bright probes that span the range of useful wavelengths and whose properties (e.g., photostability and environmental sensitivity) are advantageous for the

Table 6.1. Brightness of Fluorescent Labels

Dye	Extinction	Quantum Yield	Brightness (kilobrights)	Percent of theoretical maximum
Fluorescein	70 k	0.70	49	70%
Alexa	80 k	0.80	56	80%
Cy3	150 k	0.04	6	4%
Rcy3	150 k	0.80	120	80%
Cy5	250 k	0.300	75	30%

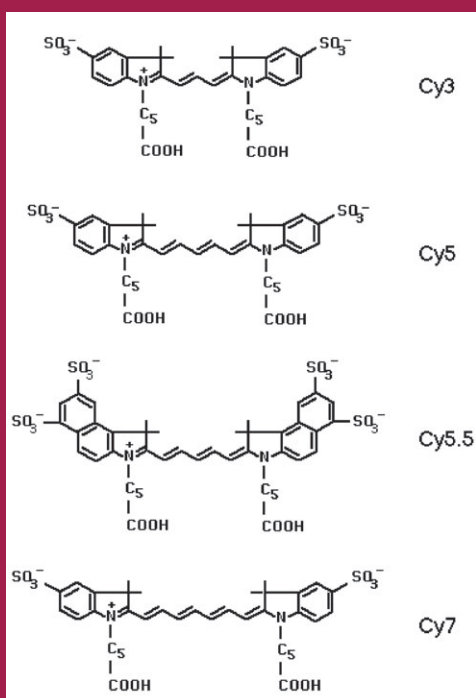


Fig 6.8. Series of cyanine dyes with an increasing number of conjugated double bonds. As can be seen in the next figure, excitation and emission wavelengths increase with the number of conjugated double bonds. [Source: Alan Waggoner, Carnegie Mellon University]

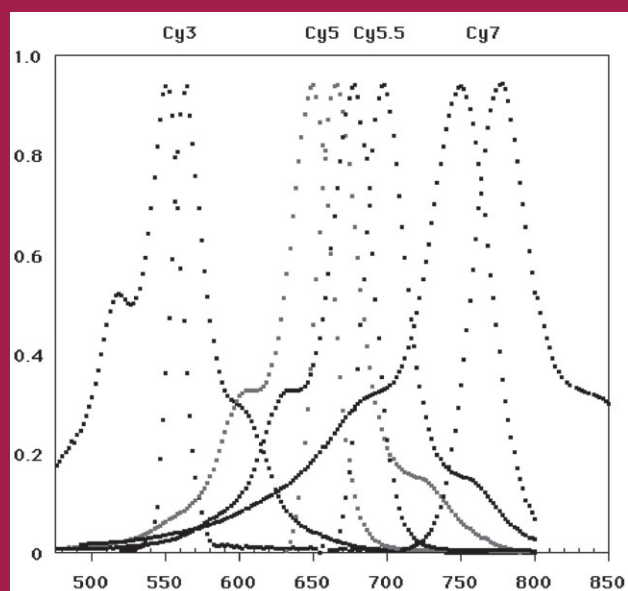


Fig. 6.9. Excitation and emission spectra shown for Cy3 through Cy7. [Source: Alan Waggoner, Carnegie Mellon University]

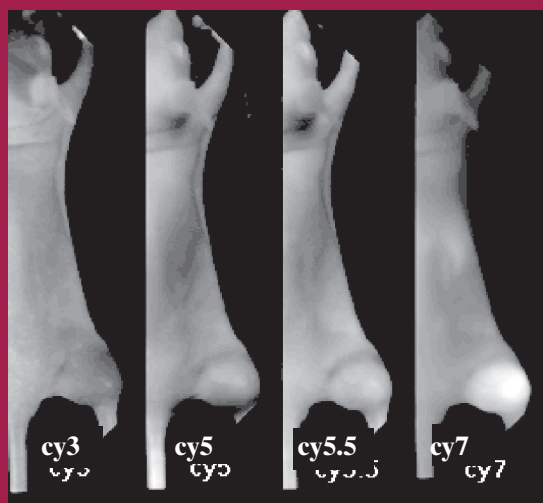


Fig. 6.10. Subsurface cancer shown as more easily detected by the antibody-conjugated Cy7 dye than other Cy dyes because the emission properties better match the tissue's optical window. [Source: B. Ballou et al., "Cyanine Fluorochrome-Labeled Antibodies In Vivo: Assessment of Tumor Imaging Using Cy3, Cy5, Cy5.5, and Cy7," *Cancer Detection and Prevention* **22**, 251–57 (©1998). Reprinted with permission from Elsevier Science]

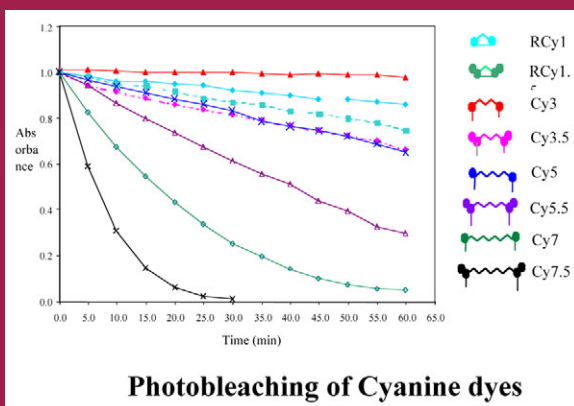


Fig. 6.11. Photostability of a series of cyanine dyes. [Source: Alan Waggoner, Carnegie Mellon University]

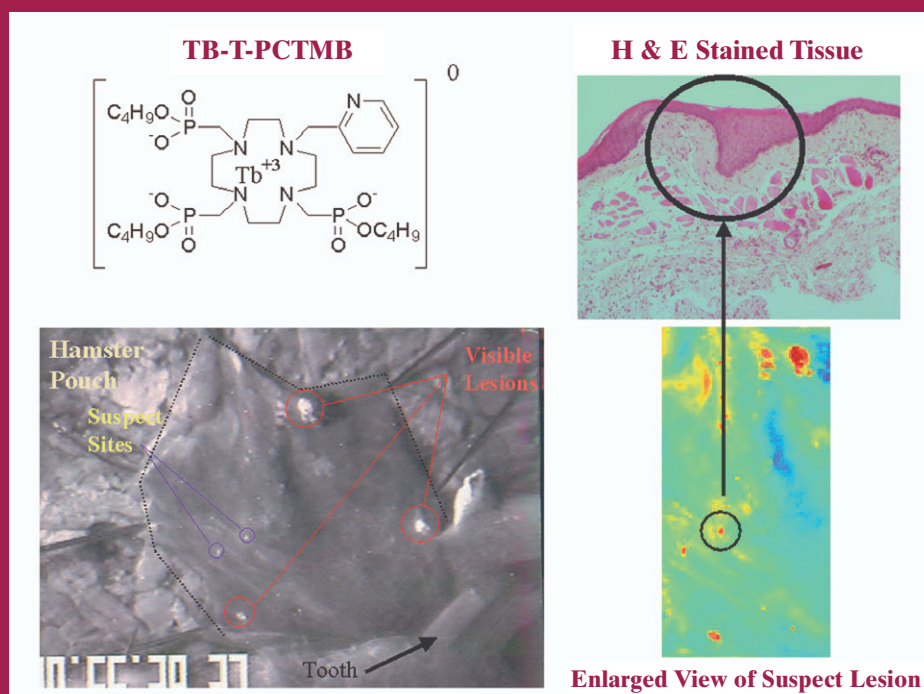


Fig. 6.12. Large spectral shifts (~260 nm) possible with lanthanide chelates because absorption is due to antennae attached to the macrocycle holding the metal, while metal ions provide fluorescence. Images of the hamster cheek pouch show that the pyridyl butyl-ester complex of Tb³⁺ has the potential to detect early-stage cancer lesions on tissue surface using simple light sources and direct visualization. [Source: Unpublished results from the Bornhop Research Group at Texas Tech University]

Environmental Sensitivity

Fluorescent labels are optimal if they are environmentally insensitive. Physiological indicators often require sensitivity to charge distributions, viscosity, solvent polarity, and conformation of their local environments. There may be opportunities for improvements in these areas.

Chemical Stability

Some fluorescent dyes, particularly at long wavelengths, are too unstable for many practical applications. Improvements are needed.

Solubility and Size

The smaller and less perturbing the fluorescent probe, the better. Solubility in aqueous systems is a challenge in core fluorophore development because of the usual requirement for conjugated ring systems. Also, fluorophores in the visible and near IR have a minimum size to absorb light at these wavelengths. Development of useful up-converting fluorophores may be possible.

Fluorescent Probe Design: Adaptation of Fluorophore to Create a Probe

Targeting

Genetic methods for creating fluorescent labels and sensors within cells need continued pursuit. Few current probes can be considered as true site-specific labels. Other methods need to be developed to introduce organic and inorganic fluorescent probes into multicellular systems. Loading probes into bacterial cells is particularly problematic (Fig. 6.13).

Figures 6.14 and 6.15 compare penetration of probes in living vs fixed multicellular systems. Targeting of biosensors to quantify cytokines and growth factors in interstitial spaces is needed. Peptide-based delivery systems and other chemical tricks sometimes prove useful for targeting.

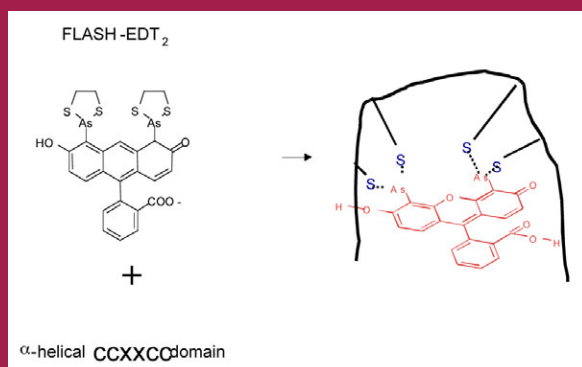


Fig. 6.13. The fluorescein arsenide FLASH-EDT₂ specifically labeling proteins containing the tetracysteine sequence CCXXCC. This provides a way to introduce a site-specific fluorescent label in proteins. [Redrawn with permission from B. A. Griffin, Stephan R. Adams, and Roger Y. Tsein, "Specific Covalent Labeling of Recombinant Protein Molecules Inside Live Cells," *Science* **281**(5374), 269–72. ©1998 American Association for the Advancement of Science]

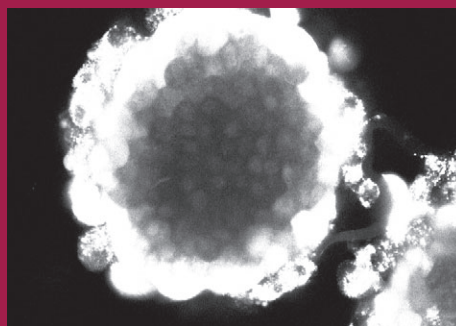
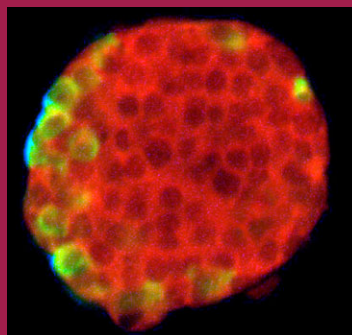


Fig. 6.14. A single optical section taken by two-photon excitation imaging through an intact pancreatic islet (~100-μm diameter) loaded with Fura-2/AM. Only the outer two cell layers incorporate the probe, while the cells in the center remain unlabeled. [Source: Dave Piston, Vanderbilt University]

Fig. 6.15. A single optical section of a fixed and permeabilized pancreatic islet. This islet has been immunolabeled with antibodies to insulin (red), which labels β cells, and glucagon (green), which labels α cells. Uniform penetration of these dyes is possible in fixed samples. [Source: Dave Piston, Vanderbilt University]



Reporter Functionality – Sensitivity to Environment

Addition of chemical groups that confer specificity to environmentally sensitive fluorophores is needed to create probes for new targets, whether they are ions, enzymes, or new chemical environments of cells.

Fluorophore Modifications

Modifications of fluorophores change solubility properties, reduce nonspecific binding, and affect targeting, penetration, and clearance of probes in cells and tissues or multicellular systems. These properties must be addressed in fine-tuning the properties of a probe instrument (Fig. 6.16).

- Considerations for fluorescent probes
- Bright, multicolor, solid-state lasers over spectrum
- IR-detector improvements as new near-IR probes are developed
- Picosecond and nanosecond excitation-detection engines as new probes appear in the 10- to 100-ns lifetime range

- Rapid, high-rejection multicolor optical filtering for multicolor fluorescence detection
- Long working distance—high NA objectives
- Environmental systems for cells and tissues
- Cheap, easy-to-use, fast, powerful software systems

Probes for Technologies Other Than Fluorescence

Plasmon-Resonant Particles

Plasmon-resonant particles (PRP) are metal nanocrystals, typically gold or silver, capable of supporting a plasmon resonance (Fig. 6.17). This feature causes them to scatter light with a narrow distribution of wavelengths. The color of the scattered light is dependent on particle size. PRPs are a million times brighter than fluorescent chromophores, and, because the optical signal results from scattering rather than an absorption and emission process, they are not susceptible to photobleaching, which makes them useful for monitoring dynamics over long time scales (hours). Their large size, however, restricts their use to monitoring dynamics in vitro.

Multicolor detection - one laser

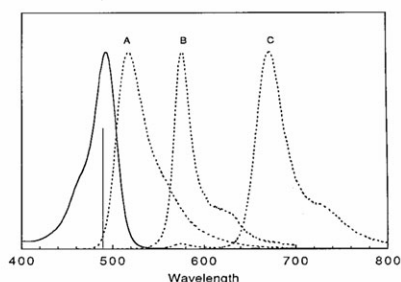
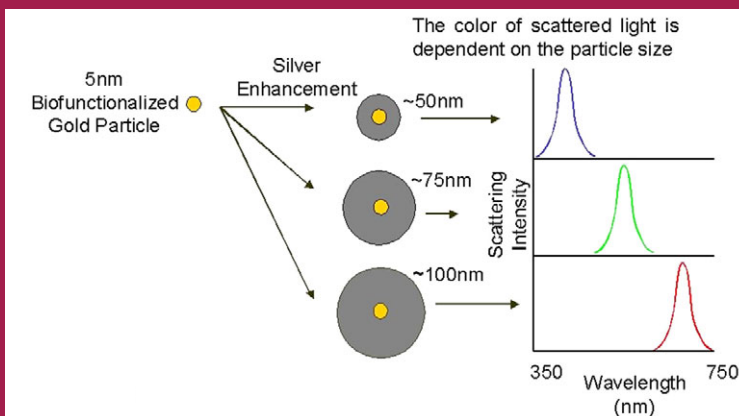


Fig. 6.16. Dyes that are excited at a single source but have emission at different, easily resolved wavelengths. An advantageous three-dye system is depicted here. [Source: Alan Waggoner, Carnegie Mellon University]

Fig. 6.17. Plasmon-resonant particles useful for monitoring dynamics over long time scales. [Source: Alan Waggoner, Carnegie Mellon University]



Rare-Earth Complexes

Rare-earth complexes have extremely long excited-state lifetimes (on the order of microseconds). This property makes them useful for time-gated imaging of biological samples. Using time-gated imaging eliminates the large background from autofluorescence and allows single molecules to be observed.

Quantum Dots

Quantum dots are small nanocrystals of semiconducting materials that display luminescent properties similar to organic chromophores but are more robust and provide longer-lived probes for monitoring dynamics over longer time scales. While quantum dots have sparked a great deal of interest and research, their implementation in the biological arena has been quite limited because of the difficulty in maintaining their robust optical properties when probes are biofunctionalized. New approaches are needed for biofunctionalizing quantum dots and novel materials for quantum dots.

Surface-Enhanced Raman Particles

SERS provide a robust signal and are easily biofunctionalized. However, their similarity in size to PRPs limits their applications to in vitro studies.

Dye-Loaded Silica Particles

Silica particles loaded with organic chromophores are robust probes. Because the chromophore is sequestered from the environment, the structures are much more resistant to photobleaching. They are somewhat smaller than the metal nanoparticles used for PRP or SERS probes; however, they are still on the order of 10 nm, which again limits their use in vivo. To avoid having the tags interfere with protein function, particles must be developed in the

nanometer to subnanometer range. Other approaches, such as the trapping of fluorochromes in molecular cages or dendrimers, may enable production of much smaller tags exhibiting the same robustness as the silica particles.

Probes for Second-Harmonic Generation

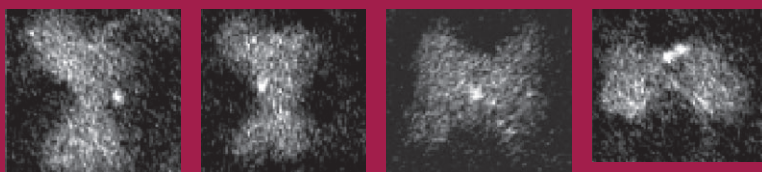
The incorporation of small particles such as polystyrene beads into a system provides a method for multiple contrast mechanisms within the same specimen. For example, one protein could be labeled with a fluorescent dye suitable for use with multiphoton spectroscopy, and another protein could be labeled with a polystyrene bead. The multiphoton fluorescence would provide visualization of one protein while the second harmonic signal generated from the beads allows the second protein to be localized.

Electron-Dense Probes

X-ray and electron microscopy require probes in the size range of 0.5 to 10 nm and composed of heavy atoms that provide high scattering contrast. Particles near molecular scale can be used to identify specific sites within molecular complexes or provide fiducial markers for particle alignment (Fig. 6.18).

Currently, the best labels are small gold clusters containing either 11 or 75 gold atoms surrounded by an organic shell with a single specific binding site for any of a variety of functional groups. These enable the localization of structures in the 2-nm range. Higher specificity can be obtained using linkers such as antibody fragments to permit localization in the 4-nm range. Larger colloidal gold particles coupled to primary or secondary antibodies are useful for identifying specific structures in a larger context. Smaller clusters, such as tetrairidium, can be used in conjunction with image averaging to give resolution in the subnanometer range.

Fig. 6.18. STEM images of freeze-dried unstained phosphorylase kinase that has undergone exchange. [Source: K. W. Traxler et al., "Direct visualization of the calmodulin subunit of phosphorylase kinase via electron microscopy following subunit exchange," *Journal of Structural Biology* **135**(3), 231–38 (2001)]



One useful aspect of electron-dense probes is the inclusion of a second contrast mechanism such as fluorescence so that the same specimen can be studied by both optical and electron microscopy. This requires careful attention to quenching by the metal particle, which can be used to advantage in producing molecular beacons. Gold-cluster contrast can be enhanced for optical microscopy by catalyzing deposition of additional metal.

Future needs include the development of additional clusters of different sizes or shapes that can be distinguished from each other and used in studies requiring multiple labels. Clusters with different metal cores would also be distinguishable in multiple labeling experiments. Another issue that must be addressed in the development of these labels is the reduction or minimization of nonspecific binding.

Summary of Needs for Probe Development

- Probes with more photostability and less toxicity, especially IR
- Probes for extreme environments that tolerate a wider range of pH and temperature
- IR probes for more color imaging and for imaging deeper into colonies, tissues, and complex multicellular systems (up to 2 mm)
- Narrower emission bands for organic dyes of multicolor analysis
- Better coatings for quantum dots
- Dyes with lifetimes in the 10- to 100-nsec range
- More genetic fluorescent probes
- Greater capability for site-specific fluorophore modification of proteins in cells
- Improved fluorescent probe-delivery systems for cells and penetration into colonies, biofilms, and tissues
- Creative probes for obtaining new kinds of cellular information
- Multisignature probes for imaging technologies other than fluorescence
- Ways to tether and orient probes on substrate
- Training support for development of probe-chemist manpower

Appendices



Appendix A: Workshop Attendees	51
Appendix B: Workshop Agenda	55
Appendix C: Imaging Methodologies for Genomes to Life Research	59
Appendix D: National Laboratory Capabilities and Imaging Technologies.....	69

Appendix A: Workshop Attendees

Imaging Workshop Attendees

Workshop Co-Chairs

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Damir Sudar, Lawrence Berkeley National Laboratory

Workshop Planners and Participants

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Ryszard Jankowiak

Argonne National Laboratory

*Ken Kemner
Lee Makowski
Jörg Maser
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Brookhaven National Laboratory

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Lisa Miller
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Alan Waggoner (Section 6 Writing Team Leader)

Cornell University

Watt Webb

East Carolina University

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Lawrence Berkeley National Laboratory

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*Bahram Parvin (Section 5 Writing Team Leader)

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*Steve Lane
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*Jim Jett
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*Cliff Unkefer

National Water Research Institute (Canada)

John R. Lawrence

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Mike Viola

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Prem Srivastava

Peter Kirchner

Roland Hirsch

David Thomassen

Consultant

Mike Knotek

Technical Support

ORISE

Mikki Dawn

Deborah Garland

PNNL

Julie Gephart

Appendix B: Workshop Agenda

Agenda for Imaging Workshop

Monday, April 15 Arrivals

Tuesday, April 16

8:00 a.m. Welcome and Charge to the Workshop Attendees

Marvin Frazier/Michael Viola

Product: A draft workshop report will be prepared at the meeting that will speak to a wide audience and provide technical input to DOE/OBER.

8:30 a.m. Review of the Schedule and Other Administrative Details

Steve Colson/Damir Sudar

Executive Summary Writing Team Formation and Charge

Computational Infrastructure Team Formation and Charge

9:00 a.m. Plenary Lectures (projecting a broad vision for the future needs and value of imaging to GTL research)

Biology Perspective 1: Ken Nealson (<http://wrigley.usc.edu>)

Biology Perspective 2: Peter Greenberg

Technology Perspective: Watt W. Webb

Noon: Lunch (catered)

1 p.m. Technical Session 1: Molecular Engines - Protein Complexes

Overview Presentation: Biology Perspective

Overview Presentation: Technology Perspective

Brief Presentations: A few one-slide presentations on biology needs and Technical Approaches

Guided Discussion

Molecular Engines Writing Team Formation and Charge

3:30 p.m. Writing Session 1

Rooms available for each writing team. (Teams can start organizing information before related technical sessions.)

6:00 p.m. Evening Reception

Wednesday, April 17

8 a.m. Technical Session 2: Intracellular and Cellular Structure, Function, and Processes

Overview Presentation: Biology Perspective

Overview Presentation: Technology Perspective

Brief Presentations: A few one-slide presentations on biology needs and Technical Approaches

Guided Discussion

Writing Team Formation and Charge

10:00 a.m. Technical Session 3: Multicellular: Monoclonal and Heterogeneous Multicellular Systems, Cell-Cell Signaling, and Model Systems

Overview Presentation: Biology Perspective

Overview Presentation: Technology Perspective

Brief Presentations: A few one-slide presentations on biology needs and Technical Approaches

Guided Discussion

Writing Team Formation and Charge

Noon: Lunch (catered)

1:00 p.m. Technical Session 4: Cells In Situ and In Vivo: Bacteria in the Natural Environment, Micro Environment, and In Vivo Systems

Overview Presentation: Biology Perspective

Overview Presentation: Technology Perspective

Brief Presentations: A few one-slide presentations on Biology Needs and Technical Approaches

Guided Discussion

Writing Team Formation and Charge

3:00 p.m. Writing Session 2: Parallel Report Writing Team Sessions

4:30 p.m. Evening Meal

6:30 p.m. Writing Session 3: Parallel Report Writing Team Sessions

Rooms available for each writing team to start at their convenience.

Thursday, April 18

8:00 a.m. **Report and Guided Discussion of Each Report Element**

Executive Summary

Molecular Engines

Intracellular and Cellular

Multicellular

Cells In Vivo

Image Data Computational Infrastructure

Probe Development

11:00 a.m. **Development of Completion Plan for Report**

Capture of Cross-Cutting Technologies, Methods, and Needs

Identification of Duplications

Refinement of Writing Plan

Assignments to Obtain Information and Figures not Available at Workshop

Noon: Lunch (not provided by meeting)

Those not participating in report writing may depart at this time.

1:00 p.m. **Writing Session 4: Parallel Report Writing Team Sessions**

(Bag lunches provided if the team wants to work during lunch.)

4:30 p.m. **Evening Meal (not provided by meeting)**

6:30 p.m. **Wrap-Up Writing and Capture of Follow-Up Actions**

Friday, April 19

Final Departures

Appendix C: Imaging Methodologies for Genomes to Life Research

Appendix C: Imaging Methodologies for Genomes to Life Research

Protein Identification and Characterization

A complete understanding of molecular machines will require information covering a wide range of dimensions and time scales. Such structural data as molecular dimensions, subunit structure, and overall shape typically are obtained with high-resolution techniques on a scale ranging from angstroms to a few nanometers. Information on dynamics and function of molecular machines, however, is usually obtained on larger spatial and shorter time scales that interrogate a molecular machine within its immediate environment. A variety of techniques can be used to access the structural and dynamic information of protein complexes. However, there is much room for improvement in several areas, including the possibility for exploitation of new phenomena.

Short-Wavelength Techniques

X-ray crystallography reveals atomic-resolution structures of subunits and small machines such as ribosomes.

X-ray microscopy is promising for the future but is not yet at a resolution to give internal details of complexes. The recent solution of the whole ribosome structure demonstrates that X-ray techniques can be extended to larger structures, but the process requires considerable effort.

Electron microscopy (EM) is well established for viewing complexes at 0.5- to 2.0-nm resolution. Particles typically are viewed by tomography either in 2D arrays or as isolated objects. If the sample is freeze-dried, quantitative measurement of electron scattering can give direct mass distribution useful for determining subunit stoichiometry or faithfulness of reassembly.

High-resolution techniques such as **transmission electron microscopy (TEM)** must be carried out on thin specimens placed in a vacuum chamber

and held stationary. **Flash-freezing** is a well-developed technology providing high-resolution 3D structures of isolated complexes and 2D arrays.

Scanning transmission electron microscopy (STEM) gives information complementary to TEM techniques by furnishing a direct link to the biochemistry. Use of metal clusters such as undecagold as labels for specific constituents makes STEM a valuable method for localizing gene products within complexes.

The resolution limit of **electron tomography** is not the microscope's resolution but the number of obtainable distinct views of the cell. Because every cell is different, an entire tilt series must be carried out on a single cell. Methods for lowering radiation damage are needed so larger numbers of images can be obtained on a single specimen. This may be possible using higher-voltage electrons (>500 keV) or by imaging specimens maintained at liquid helium temperatures.

Intact molecular machines composed of multiple proteins often are too large or too flexible to make a crystallographic approach feasible. This challenge is best suited to EM. Three-dimensional reconstructions can be calculated from images of a molecular machine taken at many different angles.

Optical Microscopy

Laser-based optical probe techniques show great promise for real-time monitoring of the motions of molecular machines. Evolving techniques are under the diffraction limits of focused light and will allow monitoring of individual molecules in vivo and in vitro. **Multiphoton and multicolor techniques** hold promise for high selectivity and simultaneous detection of multiple species. Advances in detector technology are needed to permit the real-time imaging of multiple species, making possible the simultaneous monitoring of chemical composition and molecular motion.

Multiplexed real-time measurements will uncover details of the mechanisms for building and operating molecular motors.

The following are specific needs:

- **Fluorescence-based microscopes** that provide high-speed, multicolor detection for study of the time dependence of the co-localization (complex formation) dynamics of complexes composed of multiple proteins.
- **Optical microscopes** that use nonlinear optical methods [i.e., **two-photon fluorescence**, **coherent antistokes Raman scattering (CARS)**, **second-harmonic generation (SHG)**, **third-harmonic generation (THG)**, and **sum-frequency generation (SFG)**] singly or in combination to obtain data about the structure and dynamics of molecular machines with minimal requirement for addition of probes.
- Single-molecule, distance-sensitive optical methods [i.e., **fluorescence resonant energy transfer (FRET)** and **lifetime time-polarization evolution methods**] to obtain real-time data on structural fluctuations within molecular machines under controlled conditions.
- **Surface-enhanced Raman scattering (called SERS)** to provide a fingerprint spectrum of the structure and to show correlated spectral fluctuations with molecular structure. The use of surface-enhanced approaches and predetermined spectral features correlated with the movement of interest should enable millisecond time scales over extended periods.

Scanning Probe Microscopy

Scanning probe microscopy is becoming a true workhorse for determining biological structure and function. It can be performed in situ (on live organisms, if necessary) without staining. This technique potentially provides for nanometer-scale topographic resolution. **Atomic force microscopy (AFM)** can be combined with and enhance **simultaneous fluorescence imaging**. It also can be used to image individual proteins, protein complexes, and molecular arrays (e.g., membrane proteins located in membranes). The phase-sensitive tapping mode furnishes information about chemical changes and “softness”

(compliance). Functionalized tips may be used to generate force vs displacement curves, providing the ability to obtain single-molecule bond-strength information. This capability lends itself to the characterization of protein-protein or protein-ligand interactions and tension-induced protein unfolding.

Diffraction Methods

Information about size and shape of purified structures randomly oriented in solution can be obtained by X-ray or neutron scattering. **Neutron diffraction** is particularly useful for defining the volume of water excluding space through the use of scattering in varying ratios of H₂O and D₂O. Variation of the H₂O-D₂O ratio can be used to contrast deuterated proteins, making possible the study of ligand binding, including conformational changes induced by the interaction. Wide-angle solution scattering also has the potential to provide scattering fingerprints for determination of the structural class of a protein in solution. For instance, this technique can readily distinguish between an immunoglobulin fold and a beta-barrel.

Structure Determination of Single-Protein Molecules or Complexes

The following are needs for determining the structure of single-protein molecules and complexes.

In the future we anticipate a need for laboratory-scale X-ray diffraction and electron scattering sources that will allow individual scientists to have dedicated machines for protein and protein-machine structure determination.

Rapid methods are needed for structure determination of single-protein molecules or complexes. Current methods, which are essential to identifying protein function, take years to provide a finished structure. **Nuclear magnetic resonance (NMR)** structure determination currently is limited to proteins of 40 to 60 kD, and X-ray diffraction methods require that the proteins be crystallized. Crystallization is the slowest step in the process, often taking many years of concerted effort. In addition, many proteins never crystallize; among these are the membrane proteins. Most molecular signaling involves traversing the membrane, and over 30% of all proteins are known to be tightly associated with membranes.

New biochemical and biophysical approaches will be required to identify the molecular structures of these proteins.

New developments are leading to the production of a “next-generation” beamline such as the **Linac Coherent Light Source** at Stanford University. This technology offers the promise of obtaining high-resolution (2 \AA or less) diffraction data from single-protein molecules, protein complexes of any size, viruses, and even cells without crystallization, using a coherent X-ray source that is ten times brighter than any today. To aid development of this technology, which may become available as early as 2007, supporting efforts will be needed specifically for single-molecule diffraction techniques using this beamline. These techniques involve sample preparation and introduction, design of rapid-readout detection arrays, and development of computer algorithms for combining and interpreting diffraction data from tens of thousands of molecules.

Imaging Biological Functions at the Subcellular, Cellular, and Multicellular Scales

Short-Wavelength Techniques

1. X-Ray Microscopy

X-ray microscopy today uses electromagnetic radiation with photon energies in the 250-eV to 30-keV range with corresponding wavelengths from 45 nm to 0.4 \AA . Because of this short wavelength, suboptical spatial resolution can be obtained. Several different contrast mechanisms can be used to form an image, thereby making X-ray microscopes useful for probing different properties of matter. High-resolution X-ray microscopes use Fresnel zone plates as focusing and imaging optics, therefore becoming the “enabling technology” in achieving suboptical spatial resolution.

X-ray microscopes operate second- and third-generation synchrotron sources because of the need for high-photon density in the specimen plane, high brilliance for all probe-based systems, and energy tunability for applications involving spectroscopy. Development of laboratory X-ray

microscopes is under way, but limited flux density from laboratory sources will most likely limit the spatial resolution in these systems to the level of 100 nm or slightly below.

Two classes of X-ray microscopes are used in both the soft and hard X-ray range. These are **transmission X-ray microscopes (TXM)**, which record a full image of the specimen in absorption and phase contrast; and **scanning X-ray microprobes**, which focus the coherent part of the monochromatized X-ray beam into a diffraction-limited spot and use secondary signals such as X-ray fluorescence or photoelectrons for contrast formation.

Specimen preparation for all X-ray microscopies is relatively straightforward because no vacuum is required and unsectioned specimens up to hundreds of micrometers thick can be penetrated. X-rays are ionizing radiation, however, and radiation damage is of significant concern. **Cryomethods**, therefore, have to be used at higher spatial resolution to achieve structural preservation. Cryomethods are a standard—if challenging—technique in the soft X-ray range and will be required in hard X-ray microscopes as spatial resolution improves.

Soft X-Ray Microscopy. Soft X-ray microscopy typically covers the photon energy range from 250 eV to 1 keV, with particular emphasis on the “water-window” spectral range between the electron banding energy of oxygen ($E_b = 543\text{ eV}$) and carbon ($E_b = 284\text{ eV}$). Here, water layers of 10 nm can be penetrated, and good contrast of the object’s carbon distribution is obtained. **Absorption spectroscopy** at the K-absorption edges of C, N, and O has been performed; some applications at higher energies involve L-edge spectroscopy of transition metals such as Fe. X-ray absorption maps and spectra can be quantified using the published elemental absorption length.

TXM and scanning transmission X-ray microscopes (STXM) are used in the soft X-ray region. TXM can operate at an incoherent bending magnet source of synchrotrons, while STXM requires the high brilliance of an insertion device to provide spatially coherent X rays of sufficient flux. TXMs are operated at the Advanced Light

Source (ALS) at Lawrence Berkeley National Laboratory (LBNL), BESSY in Germany, and Aarhus in Denmark. Because of their relatively simple setup, they furnish the highest spatial resolution of any X-ray microscope to date, currently around 20 nm. Immunogold labeling is a way to specifically tag certain proteins to investigate cellular functions. Tomography has provided 3D reconstructions of whole, hydrated cells. STXMs operated at the National Synchrotron Light Source at Brookhaven National Laboratory and at ALS are unique tools for spectroscopy such as C-XANES. They also are capable of using secondary signals such as photo-emission.

Hard X-Ray Microscopy. Although soft X-ray microscopes have reached a significant level of maturity, high-resolution **hard X-ray microscopes** using zone-plate optics have only recently begun operation. Their application to biological and environmental sciences is yet more novel, and significant R&D is under way to develop their potential. Hard X-ray microscopy covers the energy range from ~1 to 30 keV; in this energy range, **X-ray fluorescence** becomes a very efficient contrast mechanism, and the sensitivity that can be achieved by fluorescence mapping becomes significantly larger than what absorption contrast imaging can provide. By using K and L states, all elements in the periodic system above Na can be directly excited, including heavy elements like Hg, Pb, and U. X-ray fluorescence is a fully quantitative method.

Because X-ray fluorescence requires excitation by a focused beam, **scanning X-ray microprobes (SXMP)** are used primarily in the hard X-ray region. As in the soft X-ray range, these systems require a coherent hard X-ray source, which in turn needs the brilliance of an insertion device at a third-generation synchrotron and high electron energies of 6 to 8 GeV in the storage ring. Only three such facilities exist in the world: the Advanced Photon Source at ANL, European Synchrotron Radiation Facility (ESRF) in France, and Spring8 in Japan. A TXM for the energy range around 8 keV is set up at ESRF. As transmission contrast becomes smaller with increasing X-ray energy, a TXM for the hard X-ray range

can no longer be operated by a bending magnet, instead requiring the full photon flux of an insertion device at a high-energy synchrotron.

Very high trace metal sensitivity, on the order of 10 to 17g for transition metals, can be routinely achieved in an SXMP. Therefore, hard X-ray mapping provides a unique and powerful way to map trace-element distributions in cells and microbes. Energy-dispersive detectors allow collection of X-ray spectra of all elements below the excitation energy, so whole sequences of elements (e.g., P, S, Cl, Fe, Ni, Co, Cu, and Zn) are mapped simultaneously. X-ray spectroscopy can in principle be performed for any element heavier than Na. Particularly for Ca and heavier elements, spectroscopy at trace sensitivity can be carried out. This allows, for example, collection of XANES spectra of Cr in microbial specimens or distribution mapping of Cr subspecies in the specimen. The spatial resolution of hard X-ray microprobes is limited by the technology available for the manufacture of Fresnel zone plates. Current technologies limit the spatial resolution achievable with reasonable efficiency to ~100 nm. A significant investment is required to improve the technology to push the spatial resolution to 30 nm or better.

Applications. X-ray fluorescence mapping can be applied to trace metals such as Fe, Cu, Zn, As, Se, Cd, Hg, Pb, or U in organisms like microbes or eukaryotic cells, while simultaneously collecting distribution from P, S, Cl, K, and Ca.

Challenges. The critical element of any X-ray microscope is the zone plate used for imaging or focusing. The outermost zone width and the aspect ratio define the spatial resolution and diffraction efficiency that can be achieved. A major investment in zone-plate technology is therefore needed to improve resolution. Achieving an outermost zone of 10 nm or less, while maintaining a thickness on the range of 200 nm for soft X-rays and $>>1\ \mu\text{m}$ for hard X-rays, would enable a major breakthrough in all applications. Another challenge is X-ray tomography. In particular in the hard X-ray region, 3D imaging is not feasible at dwell times of a second per pixel. A major improvement could be achieved by maximizing the solid angle of detection using novel detectors and improving the brilliance of hard X-ray storage rings.

Electron Tomography

Electron tomography has the potential to reach the highest spatial resolution in imaging 3D structures of intact cells and allow mapping of the locations of subcellular components. Specimens can be prepared in the frozen-hydrated state, preserving the native conformation of the cell and its contents. Intermediate-voltage electron microscopes, operating at 300 to 400 kV, allow imaging of samples several hundred nanometers thick, so we can examine elongated bacteria such as *Magnetospirillum* and *Caulobacter* intact. Theoretical analyses suggest that we should be able to reach a resolution of 2 to 5 nm with such specimens. At this resolution, identification of subcellular features such as cytoskeletal filaments and nucleic acid condensates should be possible. Also, large macromolecular complexes such as ribosomes, proteasomes, and polymerases could be identified by matching the observed densities with structures of corresponding molecules that have been solved by X-ray crystallography or other techniques.

Although the ability to identify the larger protein complexes directly will provide a wealth of new information, selective labeling of components with electron-dense agents will enable localization of more components. Labeling strategies could include conventional antibody-targeted metal atom clusters. The use of quantum dots, for example, would allow correlative light microscopy, but the particle size for EM would be smaller than for current light microscopy. A problem yet to be addressed is how to get the particles into the bacteria. Evidence suggests that complexing them with a naturally imported peptide such as transferrin may work. Other labeling approaches under development include modification of a particular gene to include a photoconvertible eosin or tetracysteine peptide that can be complexed with a heavy-metal atom cluster.

Instrumentation developments are needed before the technique becomes routinely applicable. Microscopes operating at even higher voltage would be helpful in obtaining higher resolution with thicker specimens. Such developments probably will be driven mainly by the materials science community. The performance of charged coupled display (CCD) cameras used

to record images on the microscopes degrades at higher voltages, so technology must be developed to compensate for this loss in performance. The microscope's specimen stages need to be improved so they operate reliably with liquid helium cooling, eucentric rotation around the tilt axis, and rotation of the grid within the holder. Automation of the microscope operation for recording the series of images is still under development.

Specimen preparation for frozen-hydrated, whole-mount samples is fairly well developed, but improvement of the methodology for sectioning frozen samples would open up the technique to applications with multicellular samples. Finally, managing the image data sets and searching the reconstructed volume densities for patterns corresponding to known structures will be significant computational efforts.

Optical Microscopy Methods

Many imaging methods using visible or **near-infrared (called NIR)** light are a part of optical microscopy. We loosely define wavelength boundaries as the optical transmission windows of cells and common glasses (~300 nm) through the range of efficient detectors (~1700 nm). These methods vary in difficulty, from inexpensive and routine tools to major interdisciplinary efforts requiring investments at the national-laboratory scale. The accessible-distance scale ranges from the "optical limit" of about half the wavelength to the macro scale, or, in other words, from the bacterial and cellular organelle scale to sample sizes limited only by optical transparency.

Image production results from a wide variety of physical and chemical properties, providing rich information content. In favorable circumstances, the detection limit may be a single fluorescent molecule. To define a role for GTL in meeting the needs of the biological community, we may classify optical-imaging methods according to difficulty and by application. This classification also is intended to show where multidisciplinary teams of physicists, chemists, and biologists should be encouraged to work together to produce the capabilities required for the next generation of methods and instruments.

Classical One-Photon Methods

All “classical” microscopy methods use continuous or low-power light sources. The most routine methods use white-light illumination in either bright- or dark-field configurations, with the more-usual transmitted light or the less-common front-side or epillumination. Contrast may be improved by a number of physical methods including **phase contrast** or **differential image contrast (called DIC)**. This is the most straightforward of microscopy techniques and is usually a part of the everyday laboratory environment. Detector technology advanced recently by introduction of **direct electronic detection** (CCDs) to replace film, but further advances are possible in sensitivity as well as in spatial and temporal resolution.

Specific and very useful information is provided by fluorescence methods, using intrinsic or added dyes. A variety of imaging systems are available, using wide-field or confocal methods. The dyes may have either general or highly specific affinities for biological constituents of interest either within cells or in the extracellular matrix. Fluorescent dyes can be tailored to provide environmental data such as membrane potential, pH, or polarizability of the local environment. Intensity information can be useful, particularly with ratio-detection methods, but content is improved considerably by making images based on fluorescence lifetime and spectral shifts.

Although many probe molecules are available, creating improved labels is an important effort. Better photostability, improved ability to reach targets within or on live cells, lessened photo-induced toxicity, and the ability to quantify chemical messengers or environmental characteristics around the cells or within the tissue require continuing research. In general, fluorescence techniques are routinely available and do not require a national commitment.

Obtaining chemical data (location of abundant species or important minor chemical compounds) is difficult with the above techniques. A direct means for obtaining molecular information is by vibrational resonances, either by IR or by Raman. IR methods are described in the “Infrared Imaging Methods Based on Intramolecular Vibrational Modes” section below.

Raman measurements can be performed with the same equipment as fluorescence. While sensitivity is a problem, full 3D imaging can be obtained with confocal methods. In special cases, the target molecule may be detected at improved sensitivity by resonance Raman techniques. Although improved detection technologies will be useful, those for Raman and fluorescence measurements are approaching their theoretical limits, leaving better probes as the leading development opportunity.

A special imaging category is **scanning optical tomography**, which requires a pulsed light source to provide depth profiling information. While the laser must produce ultrashort light pulses, high power is not required, and obtaining 3D information useful on a tissue scale but not on a single-cell dimension is possible.

Multiphoton Microscopy

The most promising avenue for obtaining new kinds of information—and the area where funding at the national-laboratory level can provide the greatest impact—is in multiphoton methods. A wide variety of characteristics may be studied, with the general requirement of an ultrafast laser technology. Very often, an experiment may be designed to provide numerous data channels for the production of multidimensional images, with a number of useful ways to understand the complex biological sample.

Two-photon fluorescence (TPF) microscopy is the most common application. A very intense, long-wavelength laser pulse can excite fluorescent molecules at the focal region, even in the transparency window. Typically a titanium-sapphire laser producing a train of pulses in the range of 700 to 1000 nm is used to excite a wide range of species, including NADH or other intrinsic chromophores and a variety of blue-, green-, or red-emitting fluorescent probes. Both organic and inorganic species are routine. The technique can involve temporal and spectral resolution, polarization information, and (in some cases) Raman and other scattering signals. Optical penetration is much improved, and the method is intrinsically suited to 3D imaging because the signal is produced only at the focus of the laser beam. Signal detection is simplified since a confocal aperture is not required, and the

signal may be collected by epidetection. The primary development effort required for wider access of TPF methods is improved (i.e., smaller, cheaper, and more reliable) laser technology and improved optical probes.

Direct access to fluorescent amino acids within proteins is possible by **THG** (chromophores at wavelengths <300 nm).

A variety of physical characteristics are accessible by related methods. **SHG** produces a monochromatic beam at half the wavelength of the incident beam. It is a coherent process and consequently sensitive to physical properties, particularly the organization of interfaces within the distance scale of the wavelength of light. Only asymmetric structures are visible as a consequence of optical-selection rules (most cell membranes produce a negligible signal). Some ordered macromolecules such as collagen produce a very large SHG signature. Useful physical information is available by comparing the transmitted, or forward, SHG with the backscattered signal, which is insensitive to phase and consequently produces very thin structures.

Another physical property may be imaged by THG, which requires lasers with wavelengths longer than 1200 nm to provide a signal transmitted in the optical window. Such ultrafast laser sources are not routinely available.

The nonlinear microscopy analog of Raman imaging is **CARS**. Various CARS applications (i.e., epi, trans, pol, time-delayed, and BOXCars) provide particular imaging advantages, and new methods for taking advantage of them are under development.

Multifunctional Microscopy. A unique advantage of multiphoton microscopy is the possibility of combining various imaging technologies. All the signals are generated simultaneously at the focal point of the laser, often available in both the transmitted and epi directions. Fortunately, the signals may be separated and detected in separate channels. Particularly useful combinations are **CARS** and **TPF**, or **TPF/SHG/THG**, although strategies for collecting **CARS/TPF/SHG**, in both epi and transmitted directions, are available. A

rich, perfectly registered, multidimensional image data set that contains a variety of chemical, physical, and structural data may be produced.

Challenges and Needed Technologies for Multiphoton Imaging. Clearly, the laser systems for **CARS** techniques are too complex for routine operation. Technologies for improving the lasers and detection systems, needed for widespread adoption of new multiphoton imaging methods, are possible but not commercially available. Conditions for optimizing laser sources to produce the best images with tolerable signal levels will require continuing effort. Providing advanced imaging tools for routine biological research and as related imaging tools at other national-laboratory imaging facilities will be helpful for continuing progress in a wide variety of biological problems.

Infrared Imaging Methods Based on Intramolecular Vibrational Modes

Far-field infrared vibrational-imaging methods will provide an important complement to fluorescence- or absorption-based optical approaches for the study of living individual cells and colonies. The use of synchrotron radiation and lasers provides diffraction-limited spatial resolution, which could be improved further by the use of image-enhancement algorithms. Vibrational signatures are specific to molecular structure and, as such, can produce direct chemical information without the necessary use of dyes or other labels. Because of the nondestructive nature of single-photon mid-IR probes, vibrational imaging can be exploited to observe dynamic changes in living cells.

Far-field infrared vibrational imaging can be developed to examine community signaling or colony and substrate chemistries at the scale of a few microns and above. Collectively, vibrational-imaging techniques should be developed to analyze microbial response at the level of individual cells or communities to perturbations such as changes in oxygen or other substrate levels (e.g., NH_3 and metals), pH or temperature changes, and other factors that will affect critical elements of the interaction of microbes with their environment. Because such hyperspectral imaging methods can potentially yield large data sets including spectral, spatial, and temporal

dimensions, imaging-analysis methods that can properly exploit and manage such data sets should also be developed.

In addition to their application to intrinsic molecular signatures, vibrational-imaging methods could be developed to take advantage of the introduction of molecules that have unique vibrational signatures.

Thus, “tagging” pathways or structures in microbes by the use of vibrational labels such as stable isotopes or ligands (e.g., CN and CO) could allow selective detection of specific molecular components within and among microbes.

Near-Field and Scanning Probe Imaging Methods

Near-field microscopies typically are based on the spatial confinement of photons by probe-microscopy structures. They offer attractive pathways to achieving resolution beyond the diffraction limit and could potentially combine topographical imaging with chemical sensitivity. Due to their dependence on probe-microscopy techniques, near-field probes usually are limited to cell exteriors, but this restriction could be overcome with newly developed photonic force microscopes that could “grab” artificially introduced labels within a cell or cell constituent and move them to probe the cell interior.

Chemically functionalized near-field tips also could be injected through the cell membrane and positioned within the cell to measure the uptake of chemicals through the cell membrane or their diffusion within the cell.

The development of near-field imaging capabilities using narrow-band laser light to achieve 8/10 using apertures is highly desirable. Later, 8/100 could be developed using apertureless systems with scanning probes within living cells and membranes.

Magnetic Resonance Methods

Magnetic resonance imaging (MRI) and (localized) **magnetic resonance spectroscopy (MRS)** have matured as powerful methods for studying biological objects. These methods are noninvasive and nondestructive, so live objects can be examined and an object can be studied

repeatedly to follow possible changes. Moreover, in principle, the object need not be disturbed because no contrast agents are required.

With **MRI**, usually the water-density distribution is imaged, and this method also can be used to study flow and diffusion of water and other fluids in the object. With **MRS** the presence, concentrations, and spatial distributions of small-molecular-weight chemical compounds such as cell metabolites can be examined; this can assist, for example, in gaining an improved understanding of metabolic activities in the object.

Specialized instrumentation and technologies have been developed to make **MRI** and **MRS** applicable in both large- and small-scale objects, and the techniques have been applied to study objects as small as a single eukaryotic cell and as large as human patients. Also, **MRI** instrumentation has been developed that can be used in the field to study, for example, the presence of oil and the propagation of groundwater. Moreover, **MRI** has been used to study the growth of biofilms and their distribution in porous and nonporous bioreactors, and **MRS** of protons and other magnetic nuclei has been used to investigate metabolic pathways and their kinetics in microbial systems. Therefore, **MRI and MRS** are potential key techniques for examining prokaryotic cell systems of different sizes, in both the laboratory and the field.

Challenges. The main challenge of **MRI and MRS** is to improve the techniques’ notoriously low sensitivity-per-unit time, which severely limits the spatial resolution of the images. The best (isotropic) resolution now obtainable in a water image of a small (1 mm or less) cell system is about 10 μm . This resolution is reduced to $\sim 100 \mu\text{m}$ when the metabolites, which have a much lower concentration than water, are mapped. In larger samples the resolution is further reduced roughly proportionally to the sample size. This means that only relatively large microbial communities can be investigated and that the NMR sensitivity needs to be multiplied at least three times to increase the image resolution to $\sim 1 \mu\text{m}$, the minimum resolution needed to image a single microbe.

Possible ways to increase MR sensitivity are to (1) use ultrahigh magnetic fields and construct ultrasmall MR microcoils, (2) cool the MR coil and receiver system, (3) enhance the MR signal via special techniques such as dynamic nuclear polarization, (4) enhance the MR image resolution by simultaneously imaging a system with another imaging technique having a larger spatial resolution, and (5) use a combination of all the above. Moreover, ultralarge magnetic gradients could be used to avoid diffusion losses in the high-resolution images. Enhancing the sensitivity also means enhancing the temporal resolution.

Another important challenge is to improve MR information for metabolic studies. Examples of such improvements are the development of techniques to enhance spectral resolution, separate intra- and intercellular metabolites involved in the metabolic processes, and use spin-labeled chemicals to follow the spatial and temporal evolution of metabolic changes with multinuclear MRS such as ^{13}C and ^{31}P MRS.

Appendix D: National Laboratory Capabilities and Imaging Technologies

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This section is a summary of imaging capabilities and technologies available at DOE national laboratories and is not intended to be a comprehensive list. Contact names and Web sites are provided for more information on each laboratory's capabilities.

Argonne National Laboratory (ANL)

Contacts: Lee Makowski (lmakowski@anl.gov) and Jörg Maser (maser@aps.anl.gov)
Web Site: www.bio.anl.gov/resources/

X-Ray Absorption Spectroscopy

This technique provides up to 0.01-Å resolution related to the radial distances from an absorbing atom to its neighbors. The local chemical probe provides information from ~0.01 to 5.0 Å in length. Additionally, it can provide information on chemical speciation and valence state for the absorbing atom. Depending on the absorbing atom's concentration in the sample and the design of the experiment, information can be obtained at the subsecond (sometimes at the microsecond) time frame.

X-Ray Fluorescence Imaging

This technique provides information related to the spatial distribution (150-nm resolution) of elements (atomic number > ~15) in a sample; imaging is not necessarily molecular unless a specific element is associated with that molecule. Time resolution depends on the ability to flash-freeze the sample each time a 1-h measurement is made.

Microfocus Secondary Neutral Mass Spectrometer

ANL also has been developing a microfocus secondary neutral mass spectrometer called SPIRIT that is capable of desorbing molecules using either a 0.25-μm laser pulse or a 50-nm-

diameter ion beam. SPIRIT photoionizes the desorbed molecules using a VUV laser source for single-photon ionization and then mass-analyzes the photoions in a 4-m time-of-flight (TOF) mass spectrometer. Molecules can be imaged with an extremely high useful yield.

Brookhaven National Laboratory (BNL)

Electron Microscopy (EM)

Contact: Joseph Wall (wall@bnl.gov), Biology Department

Scanning Transmission Electron Microscopy (STEM)

Web Site: <http://bnlstb.bio.bnl.gov/biodocs/stem/stem.html>

STEMs, unique electron microscopes built at BNL, provide quantitative microscopy to biologists. More than just a picture, the digital STEM image provides data used in mass analysis performed by PCMASS, a computer program written by a STEM facility scientist. Individual molecules or assemblies of molecules of up to several billion daltons can be "weighed." One of the few electron microscopes in the world that can image single heavy atoms, STEM is suitable for visualizing tiny gold clusters attached as tags for biological macromolecules.

An NIH Biotechnology Resource since 1977, BNL-STEM1 conducts more than 50 active projects a year with scientists worldwide, using mass mapping and heavy-atom labeling for studies of native and reassembled complexes in the molecular-weight range of 0.1 to 1000 MDa. STEM1 is a custom-built instrument operating at 40 keV and -160EC with 0.25-nm spot size and efficient dark-field detectors capable of visualizing single heavy atoms and unstained DNA.

BNL-STEM3 is similar to STEM1 but operates at 70 keV with addition of an electron energy-loss spectrometer for elemental analysis. Operational since 2000, it is not a user facility.

Cryoelectron Microscopy (Cryo-EM)

BNL has a JEOL 2010F cryo-EM equipped with a 200-KeV field emission gun, a high-tilt stage, cryostage, 2-K by 2-K charged coupled display (CCD) camera, and STEM capability. Its planned use is for studies of membrane proteins in 2D arrays and for single-particle analysis by tomography; it also should be useful for STEM studies of frozen-hydrated specimens.

Atomic Force Microscope (AFM) and Scanning Tunneling Microscope (STM) in Air and Liquid

Contact: Stanislaus Wong (sswong@bnl.gov), Materials Sciences Department

BNL's state-of-the-art Nanoscope IIIa Multi-mode AFM allows the measurement of topographic data, viscoelastic properties, roughness, and morphological inhomogeneities as well as chemically sensitive phase information on the surfaces of samples (<5 nm routinely doable), often in simultaneous data-collection modes. Force measurements have a sensitivity of about 20 pN, and dynamic processes such as crystal growth can be measured.

Performing STM measurements is also possible with this instrument, which is capable of obtaining atomic resolution. Several probes are currently available, including conventional silicon nitride and silicon tips. A nanomanipulation setup is available for fabricating carbon nanotube tips important for high-resolution imaging.

Advanced Electron Microscopy Facility

Contact: Yimei Zhu (zhu@bnl.gov), Materials Sciences Department

BNL's Advanced Electron Beam Facility has several instruments, including the newly installed 300-kV JEOL 3000F transmission electron microscope (TEM), currently the best of its kind in the United States. It has a very bright field-emission source with a probe size down to

<0.4 nm, a high-resolution pole-piece (point-to-point resolution <0.16 nm and lattice resolution <0.1 nm) with a permissible sample tilt of $\pm 30^\circ$. It is equipped with an integrated Gatan energy filter, an electron energy-loss spectrometer, and an energy-dispersive X-ray spectrometer for high-quality imaging, diffraction, and spectroscopy.

The instrument also has analog- and digital-scanning units for spectroscopic imaging and chemical mapping for both light and heavy elements at a spatial resolution better than 1 nm and an annular dark field detector for Z-contrast imaging. An attached electron holography unit is designated to retrieve phase-sensitive magnetic and electrostatic information directly from the samples, while the heating and cooling stages (temperature range 15 to 1300°K) and versatile data-acquisition assemblage (Fuji Imaging Plate System and Gatan CCD and video cameras) allow quantitative, dynamic in situ experiments with a temporal resolution of 1/30 s.

The instrument is dedicated to materials science research for revealing crystal, electronic, and magnetic structures at nanoscale in solid state. It also is suitable for medical and biological research. We have demonstrated the instrument's ability to image boron distribution at nanometer resolution using the boron K-edge of biological samples, including tobacco mosaic virus.

Magnetic Resonance Imaging (MRI) with Contrast Agents

Contact: Charles Springer (cspringer@bnl.gov), Chemistry Department

In the context of MRI, the term "molecular" imaging means the use of paramagnetic contrast reagents (CR) targeted to specific sites on enzymes, receptors, and polynucleotides. Unlike the case for radiotracers, in MRI the CR is detected indirectly via its effect on the $^1\text{H}_2\text{O}$ nuclear magnetic resonance (NMR) signal measured. This generally requires some degree of molecular access of water to the CR. Among other features, it allows the design of CRs that are "activatable" only upon interaction with the target. Thus, they are somewhat analogous to the

“molecular beacon” fluorescent probes for optical imaging but can in principle be detected from deep within any living organism’s tissue.

The BNL Chemistry MRI group has an international reputation for elucidating the physico-chemical mechanisms operative for MR CRs in vivo. For example, BNL recently discovered that the threshold concentration for detection of CR is decreased as the magnetic field increases. This is very important in the design of CRs for crucial targets present only at very low concentrations in vivo. A BNL 4-T (Tesla) instrument for human and animal subjects soon will have a 7-T instrument for rodents, allowing the imaging of human and animal subjects with spatial resolutions easily better than 1 mm and 300 μm , respectively.

Infrared (IR) Microspectroscopy

Contact: Lisa Miller (lmiller@bnl.gov),
National Synchrotron Light Source (NSLS)

Web Site: <http://nslsweb.nsls.bnl.gov/infrared/u10b/>

Synchrotron IR light is an ideal source for spectroscopy because of the combination of its high brightness (i.e., flux density) and broadband nature. Through a 10-mm pinhole, a synchrotron source is 100 to 1000 times brighter than a conventional global source, and the broadband nature of the source is important for performing spectroscopy.

The U10B beamline at NSLS is developed for combined optical and fluorescence microscopy and IR microspectroscopy to allow chemical imaging of materials. The beamline is equipped with a Thermo Nicolet Continuum IR microscope and Magna 860 step-scan Fourier Transform Infrared (FTIR). This configuration allows simultaneous sample viewing and IR data collection, including digital video recording through 15 \times and 32 \times Schwarzschild IR objectives. Using various available beamsplitters (CaF₂, KBr, and Si) and detectors (MCT-A, MCT-B, Cu-doped Ge, and bolometer), IR spectra can be collected from 50 to 5000 cm^{-1} .

Ongoing applications of synchrotron IR microspectroscopy include examination of mechanisms of heavy-metal accumulation in plants; thin metal-oxide layers and corrosion; minerals and archaeology; protein and mineral

content in bone during osteoporosis and osteoarthritis; misfolded protein structure in Alzheimer’s disease and scrapie; enzyme structure and biopolymerization; chemical changes in single cells during various stages of apoptosis; and submillisecond folding of proteins such as cytochrome c.

Microspectroscopy and Imaging at NSLS

A unique strength of NSLS imaging capabilities is the broad range of wavelengths available from the VUV and X-ray rings. This broad spectral range allows for imaging from the far IR through the hard X-ray regimes. A number of beamlines on both the VUV and X-ray rings are used for a wide range of imaging and microprobe techniques (* indicates currently under development): diffraction-enhanced imaging (X15A); hard X-ray fluorescence microprobe and imaging (X13B* and X26A); soft X-ray microprobe and imaging (X1A); microtomography (X2B and X27A); X-ray microdiffraction (X26A, X17B and C, and X13B*); infrared microspectroscopy and imaging (U2A, U2B, and U10B); and extreme UV (called EUV) lithography (U13UC).

Broad scientific fields of research take advantage of NSLS imaging capabilities. To date, the largest user communities in imaging come from the environmental, geological, and biomedical fields; a number of users, however, also come from the materials and polymer science communities. Only a fraction of current users take advantage of the multiple imaging and microprobe techniques at NSLS.

Lawrence Berkeley National Laboratory (LBNL)

Contact: Bahram Parvin (parvin@media.lbl.gov)

Web Site: <http://vision.lbl.gov/index.htm>

Instrumentation

Synchrotron radiation-based infrared (SRIR) spectromicroscopy enables the nondestructive in vitro monitoring of specific chemical information in different compartments of individual cells without treating cells with exogenous dyes, fluorescent labels, stains, or destructive techniques. Each cell in a population thus can be studied sequentially over a period from subseconds to

months. By monitoring cells individually or within a population, detecting and probing the heterogeneity of disease onset and responses to various treatments or insults is possible. The spatial resolution is 3 to 10 μm . The development of SRIR spectromicroscopy will result in a broadly applicable and powerful research tool for the scientific community.

Wide-field deconvolution and structured light microscopy allows high-resolution, 3D fluorescence imaging. Its major strengths are (1) high sensitivity across the visible spectrum, (2) ease of recording multiple-wavelength information with minimal displacement, and (3) higher spatial resolution than conventional light microscopes (about 0.2 μm in X and Y, 0.5 μm in Z). Current camera configuration allows a maximal real-time imaging mode of 17 fps but can be upgraded with available cameras. Through collaboration with the laboratory of John Sedat at the University of California, San Francisco, plans are to eventually incorporate a structured illumination mode, which is reported to allow 0.1- μm resolution in X, Y, and Z. Structured illumination provides the basis for rapid reconstruction of 3D images, which is necessary for construction of rapid responses.

Cryo-EM provides very high resolution EM imaging and tomography but is less temporal.

LBNL also has the capacity to do X-ray cryotomography as well as multiphoton, multi-spectral imaging. Consequently, the laboratory can correlate live-cell light and high-resolution X-ray microscopy analyses on the same specimen. A soft X-ray microscope at 300 to 900 eV with 25-nm spatial resolution produces images of 1000 by 1000 pixels, 1000 photons per pixel recorded in 3 s at 517 eV, 0.2% BW.

Informatics

A collection of unique algorithms for capturing 2D and 3D subcellular structures has been developed. Labeled structures provide the basis for protein expression for large-scale image analysis. These algorithms have been applied to fixed cells imaged with fluorescence confocal microscopy.

Novel visualization, morphing, and registration techniques have been developed to track evolution of cultured colonies and their corresponding properties in time.

The visual serving optical microscopy (VSOM) system quantifies physiological responses as a function of external exposures and uses them to elicit a specific response by manipulating external exposures. Strictly for live-cell studies, VSOM can monitor hundreds of cells simultaneously for statistical validation, tracking each cell spatially and measuring uptakes and retention factors for each desirable component. Plans are under way to use these physiological responses to estimate parameters such as kinetic orders and rate constants.

An imaging bioinformatics system can track distant features and correlate events and responses across genetically altered cell lines or the same cell line at different times. A data model derived from multiple experimental protocols couples experimental annotations to images and their derived features for hypothesis testing and information retrieval. The system, named BioSig, is coupled to VSOM and protein-expression studies in fixed cells.

DeepView is an extensible open-system architecture for coupling imaging instruments with computational infrastructure in distant online collaborations. The system, which provides for both synchronous and asynchronous collaboration, has been integrated with five electron and optical microscopes.

Lawrence Livermore National Laboratory (LLNL)

Contacts: Rod Balhorn (balhorn2@llnl.gov), Stavros Demos (demos1@llnl.gov), Chad Talley (talley1@llnl.gov), Tom Huser (huser1@llnl.gov), and Steve Lane (slane@llnl.gov)

Optical Microscopy

LLNL has high-speed, 3D, multiwavelength (one 4-wavelength 3D stack every 0.5 to 1 s) optical microscopy capabilities for imaging chromosomal motion, signal transduction, and time-structure analysis (250-nm resolution). The

optical microscope, contained in a temperature-controlled environment ideal for live-cell imaging, allows for automatic tracking of live samples during data collection [J. Trebes, LLNL, and J. Sedat, University of California, San Francisco (UCSF)].

Electron Microscopy

LLNL can perform EM of large molecular complexes (via single-particle reconstruction or EM tomography) (0.5- to 1.0-nm resolution) and structural analysis of large molecular assemblies that are impossible to crystallize (J. Trebes, LLNL, and J. Sedat, UCSF).

X-Ray Microscopy

X-ray microscopy of in vitro cellular structures is available through synchrotron- and laser-based X-ray microscopes for high-resolution, 3D imaging of subcellular structures in both frozen and hydrated states (30-nm resolution) (J. Trebes, LLNL).

Hyperspectral Imaging and Microscopy

Hyperspectral imaging and microscopy of cells via polarization, light scattering, and autofluorescence (250-nm resolution) are available for imaging tissue and single cells using sequential, multiple-contrast techniques within a matter of seconds (S. Demos, LLNL).

Atomic Force Microscopy

AFM is available for imaging single virus particles, proteins, and biomolecular complexes (1- to 3-nm resolution) (J. De Yoreo, C. Orme, A. Noy, and R. Balhorn, LLNL). High-resolution force microscopy involving carbon-nanotube tips can resolve individual domains in proteins and is easily used in vitro. Smart-probe AFM using chemically modified tips can monitor changes in cell surface or receptors upon stimuli (10-nm resolution) (R. Balhorn, A. Noy, and T. Huser, LLNL).

Single-Cell Microscopy

Capabilities at LLNL include single-cell Raman microscopy to obtain information about composition, structural changes, and changes upon stimuli via either single laser source or coherent anti-Stokes Raman spectroscopy (CARS) imaging (250-nm resolution) (R. Balhorn, T. Huser,

and S. Lane, LLNL). Single-cell Raman imaging based on confocal microscopy is available with 300-nm spatial resolution. This instrument can generate images of single living cells at specific Raman lines with CARS or conventional micro-Raman scattering and can obtain full Raman spectral imaging of subcellular compartments.

Single-molecule imaging techniques include fluorescence inside cells via genes with green fluorescent protein (GFP) tag to watch expression under different conditions or morphogenesis, surface-enhanced Raman spectroscopy (250-nm resolution) (R. Balhorn, A. Noy, C. Hollars, C. Talley, S. Lane, and T. Huser, LLNL), and fluorescence and surface-enhanced Raman microscopy and spectroscopy based on a confocal microscope with a spatial resolution of 300 nm.

Other Capabilities

- Ion-probe microscopies of living cells (PIXE, 1000-nm resolution), STIMS, nanosecond ion mass spectrometry (SIMS) (50-nm resolution), and single-cell X-ray absorption near-edge (called XANES) that provide material-dependent 2D (PIXE) and 3D SIMS maps of samples (R. Balhorn).
- Near-field scanning microscopy (50- to 100-nm resolution) for simultaneous topographic and subwavelength imaging (T. Huser, C. Hollars, and C. Talley).
- Single DNA-manipulation and video-imaging system (250-nm resolution) with an optical trap that manipulates single DNA molecules in multisolution flow chambers (R. Balhorn).
- Advanced fluorescence microscopy (250 nm) with fluorescence lifetime imaging; two-photon excitation imaging; and multiwavelength video imaging of single molecules in flow channels (T. Huser, C. Hollars, C. Talley, O. Bakajin, and S. Lane).
- Novel labels for imaging based on semiconductor quantum dots and gold and silver light-scattering nanoparticles (S. Lane, T. Huser, G. Galli, and G. Fox).

Los Alamos National Laboratory (LANL)

Contacts: Robert Donohoe (rjd@lanl.gov), Larry Hersman (hersman@lanl.gov), and Judith Maurant (jmaurant@lanl.gov)

Molecular-Imaging Capabilities

- **IR focal plane array (MCT) microscope (Spectra Tech)** coupled to a step-scan FTIR for continuous-wave (CW) and time-resolved molecular imaging (a full interferogram is created at each pixel). The spatial resolution currently is twice the diffraction limit ($\sim 10\ \mu\text{m}$), and coupling of the step-scan bench to a pump-and-probe trigger experiment can yield microsecond time resolutions. Current work aims to improve the S/N by several methods but has not yet explored tuning-range (called TR) capabilities, which have been used with the step-scan bench on other occasions.
- **Epi-CARS and two-photon fluorescence microscope** based on two synched mode-locked titanium:sapphire lasers. Spatial resolution is roughly 200 nm, and we have used this instrument to examine the uptake of fluorescent beads and GFP-labeled bacteria by white cells. The independent tuning of the two lasers allows full “fingerprint” vibrational CARS imaging, although we must first overcome difficulties in the synchronization apparatus. Conventional CW lasers also are available for typical fluorescence microscopic measurements.
- **Near-field fluorescence imaging.** This capability is using a wave guide to collect near-field fluorescence from quantum dots. The spatial resolution is roughly 50 nm. There is no time-resolution capability as yet.
- **Conventional Raman microscope** using a Zeiss inverted-axis microscope coupled to CW argon and titanium:sapphire lasers. Detection of hyper-spectral images is based on holographic single-stage spectrographs with CCD cameras. Spatial resolution is roughly diffraction limit (500 to 300 nm), and images take from minutes to hours to collect.

- **Development of radiopharmaceuticals** for imaging of target organs ($\sim 3\text{-mm}$ spatial resolution) using positron emission tomography (called PET) technologies.
- **Single-molecule detection.** This is not so much an imaging capability per se as a capability that uses spatial and temporal resolution to detect single molecules and monitor their dynamics, including fluorescence decay.
- **Data analysis.** Genetic learning algorithms and multiscale, multiresolution algorithms (often based on wavelet transformations) are capable of improved feature extraction compared with PCA and SVD methods.

Oak Ridge National Laboratory (ORNL)

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Imaging Technologies

Molecular-Recognition Force Microscopy (MRFM)

MRFM provides molecular-level spatial resolution of proteins present on cell surfaces. In addition, this technique can be combined with optical techniques to characterize intracellular components.

Topographic and Electrostatic Imaging of Single Molecules

This technique can measure the topographic and electrostatic properties of single molecules in real time. As an example, photovoltages from single-molecule Photosystem I reaction centers have been measured both on atomically flat gold surfaces and in room-temperature aqueous environment. The resolution of the electrostatic maps enabled us to measure the fine structure of the ferredoxin docking site at the reducing end of Photosystem I.

Nonlinear Photonic Scanning Microscopy (NPSM)

NPSM is used with simultaneous fluorescence spectroscopy of single molecules having a lateral resolution of 2.9 nm and a normal resolution of

0.1 nm. Using two-photon fluorophores, the nonlinear tunneling provides signals acquired without the usual problems of fluorophore decay.

Scanning Tunneling Microscopy (STM)

ORNL has developed a photon STM that can provide optical images and localized spectroscopy (in air and fluid) of biomaterials with a spatial resolution of 40 nm.

Atomic Force Microscopy

The spatial resolution ranges from subnanometer to a few nanometers for AFM-based techniques.

Novel Scanning Microscopes

ORNL has modified conventional scanning probe microscopes (STM and AFM) to yield structural, physical, and chemical information not readily available in conventional scanning microscopes. The spatial resolution ranges from subnanometer to tens of nanometers depending on the basic physics involved. In addition, most of these techniques can be used under solution. For example, microcantilever tips modified with biological probes can be used to locate the receptor sites on cell surfaces with nanometer precision (chemical force imaging). Combining chemical force information with phase-enhanced topography can create a chemomechanical topography of cell surfaces and biomolecules.

Optical Nanosensors for Single-Cell Analysis

Nanobiosensors are based on submicron optical fibers for in situ intracellular measurements of single-cell, antibody-based nanoprobe with spatial resolution of 30 nm (probe-tip diameter) and temporal resolution of nanoseconds (fluorescence lifetime).

Multispectral Imaging (MSI)

MSI is accomplished using an acousto-optic tunable filter (AOTF) coupled to an optical microscope. This imaging modality can visualize molecules based on many different spectroscopic techniques (e.g., fluorescence, phosphorescence, reflection, and Raman spectroscopy), thus providing real-time, in vivo information on electronic as well as vibrational information for any type of molecule. It has a spatial resolution of 250 μm and a temporal resolution of nanoseconds or shorter.

Near-Field Scanning Optical Microscopy (NSOM) and Atomic Force Microscopy

This technique is capable of monitoring fluorescence or Raman signals as well as topography. These two modes will provide information about the electronic, vibrational, and rotational structures of the molecules. NSOM has a spatial resolution of ~ 50 nm, and AFM has nanometer-to-subnanometer resolution.

Two-Photon Fluorescence Imaging (TPFI)

TPFI is capable of monitoring the fluorescence signal from 3D objects with a theoretical spatial resolution smaller than the diffraction limit of light. This is particularly useful for in vivo measurements of the electronic and molecular properties of molecules that have been immobilized in a 3D matrix. The system will provide resolution capable of approaching the molecular level, and its temporal resolution will be on the nanosecond regime or smaller.

UV Resonance Raman Microscope

This system can image molecules based on their vibrational properties. In addition to conventional Raman microscope features, the UV resonance Raman microscope is particularly well suited for stimulating the resonance Raman vibrational modes of macromolecules such as proteins. This feature enables highly sensitive detection while minimizing fluorescence background. An intensified CCD offers 5-ms acquisition times in the continuous-running mode. Special gated-mode imaging can be achieved with acquisition times as low as 5 ns.

Surface-Enhanced Raman Scattering (SERS) Microscopy

The Raman-based microscope can be used with SERS-inducing media for dramatically improved sensitivity. The SERS process typically can enhance Raman scattering signals 10^6 to 10^8 times. Temporal, spectral, and optical resolutions of the Raman microscope are not significantly affected by the SERS effect.

Surface-Enhanced Raman Scattering Nanoprobes

This system offers the spectral and chemical selectivity of SERS with highly localized and position-controlled detection. This regime

includes an inverted microscope equipped with probe micromanipulators, an AOTF and a CCD. Optical fibers can be produced with extremely small tips (e.g., 100 nm) through special tapering processes implemented in our laboratory. The intensified CCD offers 5-ms acquisition times in the continuous-running mode. Special gated-mode imaging can be achieved with acquisition times as low as 5 ns.

Confocal Raman Imaging

This scanning-based mode of imaging is appropriate for applications requiring resolution at the diffraction-limited level but for which a high level of temporal resolution is not a key factor. Furthermore, samples should be distributed in a planar format. The pinhole-based optical system virtually eliminates optical aberrations induced by lens imperfections, yielding optical resolution at the diffraction limit.

Pacific Northwest National Laboratory (PNNL)

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Cellular Observatory Instrumentation

PNNL's cellular observatory houses a collection of unique instruments:

- **CARS and two-photon confocal combined microscope.** For visualizing molecules based on their vibrational properties and simultaneously by their fluorescent properties, CARS can visualize selected molecular species, such as lipids and deuterated compounds. Spatial resolution is 0.3 μm .
- **AFM-enhanced fluorescence-imaging microscopy.** This novel nanoscale-characterization combined microscope uses the optical or AFM approach and a high-sensitivity far-field microscope to provide unperturbed measurements of reaction rates at sites that have been characterized by AFM imaging.

- **Single-molecule spectroscopy and imaging microscopy.** This instrument does simultaneous structural and spectroscopic analyses of single biomolecular complexes and their reaction (interaction and association) rates, providing insight into the relationship between structure and function of cell-signaling proteins and enzymes. Information collected from these approaches is typically lost or hidden in measurements using current conventional ensemble-averaged methodologies.
- **Single-molecule patch-clamp–optical confocal imaging microscopy.** This combines a confocal scanning linear and nonlinear optical microscope with state-of-the-art patch-clamp technologies. The instrumentation significantly enhances the diagnostic and investigative capabilities of both methods in characterizing ion channel and receptor dynamics and mechanism in a living cell.
- **High-speed confocal microscope for 3D fluorescence resonance energy transfer (called FRET) in living cells.** The microscope combines a Nipkow-disk–based confocal scanning head with dual Gen III–intensified CCD cameras and a fast AOTF coupled to a high-powered argon: krypton (called Ar-Kr) laser system. High-resolution images from both cameras (1000 \times 1000 \times 12 bits) are simultaneously captured and spooled to high-speed RAID drives at 30 fps. XYZ scanning allows 3D images to be rapidly acquired, and custom software provides for FRET and ratiometric analyses to be performed in near-real time.

Magnetic Resonance Microscopy

Several MRI capabilities are available at PNNL. These microimaging systems include

- Varian Unity + 7T vertical wide bore (89 mm)
- Varian Unity + 12T vertical wide bore (89 mm)
- Vertical Bruker Biospin 12T wide bore (89 MM)

All these spectrometers are equipped with a variety of imaging probes optimized for MR microscopy of samples varying in size from 200 μm to 4 cm, probes for (respiratory-gated) in

vivo MRI and spectroscopy of live mice and other mammals of a similar size, and a combined confocal and magnetic resonance microscope, as described in the section regarding the cellular observatory.

Several probes are equipped with sample chambers that can be made part of a perfusion system, so that live biological cell systems and the dynamics of cellular processes can be studied.

Multisensor Image Registration

Novel mathematical methods and algorithms are being developed for spatially coregistering 2D and 3D images of cellular and tissue structures and dynamic biological processes. These are being adapted from methods originally developed for registration of multisensor satellite imagery. Image data are obtained by several imaging modalities including confocal microscopy, NMR, and CARS.

Feature Extraction and Object Classification

PNNL is developing a suite of statistically based methods as well as analysis tools to detect locations of change and extract feature in fused, multisensor imagery. Two new methods (inverse kriging and conditional spectral mixing) are applied to imagery to improve the spatial resolution of image features. Statistical techniques are then used to identify and classify features or objects within the image. Methods for detecting and quantifying areas of change between successive images also are being developed. Taken together, these tools enhance analyses with improved capabilities to identify and classify features and monitor changes in multisensor images.

Three-Dimensional Visualization and Analysis Interaction Systems

Innovative 3D visualization and human-interaction technologies are being developed that greatly enhance the ability of scientists to interact with and understand biomedical imagery. New 3D display and “augmented reality” visualization technologies enable scientists to view imagery in 3D in an interactive manner. The core of this technology is the “Human-Interaction Workspace,” a tabletop display coupled with

algorithms that enable human gestures to control the image display. The intent of this technology is to enable scientists to gain deeper and more rapid insight into complex biological processes.

Parallel Computing Environment for Imaging Science

Decreasing the time to solve many digital image-processing tasks using parallel computing methods can also decrease the number of tasks. Our current research is to develop a working environment that is scalable, portable, and with near real-time image processing, steering, and dynamic load balancing. We have been able to achieve increased performance by processing a 300-MB image file in less than 6 s. Additionally, the system can display 9.2 Megapixels (24-bit) with full real-time interaction.

Image-Based 3D Reconstruction and Modeling

NWGrid, a 3D mesh-generation system under development, allows users to perform image segmentation, mesh generation, and mesh optimization on volume digital-image data. These parallel algorithms do 3D image reconstruction and feature extraction to produce geometry from which computable volume meshes are generated. We have successfully modeled a lung and a rat’s respiratory system.

Architecture for Cross-Domain Image Analysis

Although image analysis is a key element of a wide range of research domains, there are no standards for interoperability among domains. Each domain tends to use a different set of software tools and formats. Even within a given domain, there often are a variety of image formats and tools, many of which do not “play well together.” We have designed an extensible open architecture that supports interoperability among a broad range of tools and formats. The architecture includes configuration and deployment management that can extend beyond a single enterprise to support remote collaborators as well.

Novel Interactions and Visual Analytics

The effective exploitation of complex, multidimensional image data is a nontrivial challenge. We are developing visualization tools and new interactive methods to support the collaborative analysis of image data. This includes such techniques as the use of augmented reality (AR) to support 3D interactions with data, as well as remote collaboration via AR-based telerepresentation of collaborators, and direct user interaction with data using hands instead of a mouse.

Segmenting 3D Volumetric Image Data

The digital data viewer (DDV) provides an interactive interface for segmenting, comparing, and overlaying 3D volumetric-image data. The data may be a 3D stack of 2D slices (e.g., with MRI scans, confocal images, or TEM images) or from any source of volumetric data. Segmentation rules are propagated through volume regions. Algorithms for image enhancement, smoothing, and correction of distortion errors are included. Fast, robust isosurface and surface-smoothing algorithms are also part of DDV.

Sandia National Laboratories (SNL)

Hyperspectral Imaging

Contact: David Haaland (dmhaala@sandia.gov)

SNL has designed and built a hyperspectral-imaging system for DNA microarrays, cells, and other biological systems via fluorescence emission excited in the visible region with 10- μm spatial resolution. The spatial resolution can readily be extended to the diffraction limit. At least three laser wavelengths are available for excitation. The system uses a sensitive electron-multiplying CCD detector and therefore is ideal for low-light imaging applications. Because an entire emission spectrum is collected rather than a discrete band of light, multiple and even overlapping emissions can be monitored simultaneously and separated from one another. When combined with our multivariate curve-resolution software, pure fluorescence spectra of emitting species can be discovered and quantitative spatial maps of the fluorescing species can be obtained, even when no standards are available.

Multivariate Image Processing

Contact: David Haaland (dmhaala@sandia.gov)

SNL's team of researchers has developed and improved multivariate methods for quantitative and qualitative analyses. These methods, which are applicable to a wide variety of spectral data, allow the extraction of more information than is possible with conventional image analysis. Recent work with multivariate curve resolution (MCR) has shown the techniques to be very powerful in extracting highly overlapped spectral signatures from hyperspectral images of microarray gene-expression data and yeast cells expressing GFP. MCR accurately identifies the spectral species and determines the concentration of each species in every pixel even when no standards are available, thus improving quantitative accuracy and providing correction for impurity and background-emission sources.

Macro- and Microscopic FTIR Imaging

Contact: Christine M. Wehlburg (cmwehlb@sandia.gov)

SNL's Biorad Stingray FTIR Hyperspectral Imager uses a 64- by 64-pixel array detector to capture hyperspectral image cubes of either a macro (4- by 4-mm) or micro (400- by 400- μm) sample area over the frequency range of 950 to 3800 cm^{-1} , which is the typical IR "fingerprint" region of the mid-IR spectral region. The spatial resolution of the 64- by 64-array detector is $\sim 60\ \mu\text{m}$ in macro mode and approaches the IR diffraction limit of $\sim 6\ \mu\text{m}$ resolution in the micro mode. Together with sophisticated multivariate calibration and classification methods, we have addressed a variety of materials analysis, aging, and characterization problems.

Atomic Force Microscopy

Contact: Alan Burns (aburns@sandia.gov)

SNL has two AFM instruments dedicated to imaging biological interfaces such as membranes in fluid environments. The first is a Digital Instruments Multimode with assorted scanners that have a range up to 120 μm . The second instrument, which has an inverted microscope base for simultaneous fluorescence and AFM

imaging, uses a piezo plate for sample scanning. Near-field techniques are employed to push the optical resolution to <100 nm. For both instruments, the AFM topographic resolution is ± 1 nm lateral and 0.1 nm vertical.

Electron Microscopy

Transmission Electron Microscopy

Contacts: Tom Headley (tjheadl@sandia.gov) and Paul Kotula (pgkotul@sandia.gov)

SNL has several TEM systems for materials characterization. The Philips CM30 provides low Z, full spectral (elemental) imaging of energy-dispersive spectrometer (EDS) and EELS signals. It operates at 300 kV with 0.14-nm lattice imaging and 0.23-nm point-to-point resolution on electron-transparent samples. SNL also has an FEG-TEM providing phase imaging via spectral imaging of EDS and EELS signals at interfaces. This instrument also operates at 300 kV with 0.19 pt-to-pt resolution.

Scanning Electron Microscopy (SEM)

Contact: Joe Michael (jrmicha@sandia.gov)

SNL can provide high-resolution imaging via several different SEM systems. SEM capabilities have been used to characterize the microstructure, elemental composition, and morphology of bugs, dust pollen, ash, and particles. Capabilities include SE 1.5 nm at 30 kV, BSE 10 nm at 5 kV, and FIB/SEM with dual column (FEG column resolution, 2.0 nm; LMIS ion column resolution, 5.0 nm); sample chamber permits viewing low vacuum for charging or wet samples, large specimen capacity (150 × 150 × 150 mm and <5kg), and automated image analysis. Data and images are available via the Internet to the customer's desktop.

Time-of-Flight Secondary Ion Mass Spectrometry (SIMS)

Contact: Tony Ohlhausen (jaohlha@sandia.gov)

TOF-SIMS is a surface analytical technique capable of identifying molecular and elemental species at the PPM-PPB level. Because it is a mass spectral technique, TOF-SIMS is well suited for organic identification based on the unique spectral “fingerprint” caused by fragmentation of

the analyzed species. It is capable of distinguishing elemental isotopes and detecting all elements on the periodic table. The TOF-SIMS instrument at Sandia can perform depth profiles at a submonolayer depth resolution and ion imaging at 0.25- μ m lateral resolution. Typical uses of TOF-SIMS include monolayer and particle analysis, molecular-weight distribution analysis of polymers, contamination analysis, and aging studies of surfaces. Typical substrates include metal coupons, silicon wafers, paper, polymer films, ceramics, and biological specimens.

Optical Microscopy

In addition to the specialized techniques listed above, SNL has multiple traditional optical microscopes available for imaging microbes, cells, and tissues with various modes of contrast. These include fluorescence emission, confocal fluorescence emission, and phase contrast.

Thomas Jefferson National Accelerator Facility

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X-Ray Microscopy

This subcellular imaging method provides elemental image maps of carbon, oxygen, calcium, and other elements in specific chemical states. The spatial resolution is 300 nm, and the time resolution is 1 min.

Far-Field IR Microspectroscopy

This subcellular novel imaging method uses specific protein, lipid, mineral, and other specific vibrational modes as a contrast mechanism. The technique is enabled through the use of bright synchrotron radiation. Spatial resolution is 3 μ m. Temporal resolution is typically 1 s, but special pump-probe techniques can provide temporal resolution to subpicosecond time scales.

Near-Field IR Microscopy

This imaging method uses specific protein, lipid, and other specific vibrational modes as a contrast mechanism. The technique is enabled by a unique, tunable free-electron laser. Spatial resolution is 10 to 100 nm, and temporal resolution is as high as 1 ps.

Pulsed High-Contrast X-Ray Imaging

Femtosecond X-ray pulses are used to provide phase and absorption contrast in X rays of tissue. Spatial resolution is 1 mm. Temporal resolution is subpicosecond.

Terahertz Imaging and Spectroscopy

Submillimeter waves are used to recognize specific proteins as an image-contrast mechanism. Examples are basal-cell carcinoma screening and early dental-cavity detection. Spatial resolution is 0.3 mm, and temporal resolution is 1 s; with pump-probe switching, however, dynamics at the picosecond time scale can be imaged.

X-Ray Diffraction (XRD)

Jefferson Lab provides XRD of macromolecular samples such as proteins, with a spatial resolution of 2 Å and temporal resolution of 10 s.

Magnetic Resonance Imaging

Jefferson Lab has the capability to produce unique MRI imaging molecules, an example being the use of the free-electron laser to encapsulate

gadolinium in fullerenes as well as to functionalize the cage complex with a water-soluble organic group. Radioactive elements such as holmium also can be encapsulated as tracers or anticancer agents.

Large-Scale Computing and Data-Handling Capabilities

These capabilities, developed for nuclear physics, can be readily adapted. They include high-performance systems for data acquisition and control; petabyte-scale systems for data storage and management; geographically distributed software infrastructure for data analysis (advanced data grid); and secure, high-speed networking. Such capabilities and corresponding expertise at Jefferson Lab could be leveraged to benefit an advanced medical-imaging program that would include centralized data storage (disk and tape), Web-based data serving with strong authentication, and assistance in designing and deploying a fully distributed infrastructure for data ingestion and export.



Program-Planning Workshops for Genomes to Life

A series of program-planning workshops has been held to help plan and coordinate Genomes to Life. Meeting reports are placed on the Web as soon as they become available (<http://DOEGenomesToLife.org>). To learn more about the program, please see the Web site or use the contact information on the inside front cover for Marvin Frazier or Gary Johnson.

2001 GTL Workshops

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|-----------------------|--|
| June 23 | Role of Biotechnology in Mitigating Greenhouse Gas Concentrations |
| August 7–8 | Computational Biology |
| September 6–7 | Visions for Computational and Systems Biology |
| December 10–11 | Technology Assessment for Mass Spectrometry |

2002 GTL Workshops

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|----------------------|-------------------------------------|
| January 22–23 | Computational Infrastructure |
| March 6–7 | Computer Science |
| March 18–19 | Mathematics |
| April 16–18 | Imaging |

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